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(54) Title: NOVEL LOW DENSITY LIPOPROTEIN BINDING PROTEINS AND THEIR USE IN DIAGNOSING AND TREATING ATHEROSCLEROSIS

(57) Abstract

Isolated polynucleotides encoding novel polypeptides which are capable of binding to native and methylated LDL (low density lipoprotein), the isolated polypeptides, called LBPs (LDL binding proteins), and biologically active fragments and analogs thereof, are described. Also described are methods for determining if an animal is at risk for atherosclerosis, methods for evaluating an agent for use in treating atherosclerosis, methods for treating atherosclerosis, and methods for treating a cell having an abnormality in structure or metabolism of LBP. Pharmaceutical compositions and vaccine compositions are also provided.

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NOVEL LOW DENSITY LIPOPROTEIN BINDING PROTEINS AND THEIR USE IN DIAGNOSING AND TREATING ATHEROSCLEROSIS

Field of the Invention

This application claims the benefit of U.S. Provisional Application No. 60/031,930 filed November 27, 1996, and U.S. Provisional Application No. 60/048,547 filed June 3, 1997.

This invention relates to novel polypeptides (LBPs) which bind to low density lipoprotein (LDL), polynucleotides which encode these polypeptides, and treatments, diagnoses and therapeutic agents for atherosclerosis.

Background of the Invention

Atherosclerosis is the principal cause of heart attacks and strokes. It has been reported that about 50% of all deaths in the United States, Europe and Japan are due to atherosclerosis. Atherosclerotic lesions in the arterial wall characterize atherosclerosis. Cholesteryl esters (CE) are present in these atherosclerotic lesions. Low density lipoprotein (LDL) has been shown to be the major carrier of plasma CE, and has been implicated as the agent by which CE enter the atherosclerotic lesions.

Scattered groups of lipid-filled macrophages, called foam cells, are the first visible signs of atherosclerosis and are described as type I lesions. These macrophages are reported to contain CE derived from LDL. The macrophages recognize oxidized LDL, but not native LDL, and become foam cells by phagocytosing oxidized LDL. Larger, more organized collections of foam cells, fatty streaks, represent type II lesions. These lesions further develop into complex lesions called plaques, which can result in impeding the flow of blood in the artery.

It is widely believed that accumulation of LDL in the artery depends on the presence of functionally modified endothelial cells in the arterial wall. It has been reported in animal models of atherosclerosis that LDL, both native LDL and methylated LDL, accumulates focally and irreversibly only at the edges of regenerating endothelial islands in aortic lesions, where functionally modified endothelial cells are present, but not in the centers of these islands where endothelial regeneration is completed. Similarly, LDL accumulates in human atherosclerotic lesions. The mechanism by which the LDL accumulates focally and irreversibly in arterial lesions has not heretofore been understood.

Summary of the Invention

It is an object of the invention to provide polypeptides which bind to LDL.

It is yet another object of the invention to provide a method for determining if an animal

is at risk for atherosclerosis.

It is yet another object of the invention to provide a method for evaluating an agent for use in treating atherosclerosis.

It is yet another object of the invention to provide a method for treating atherosclerosis.

Still another object of the invention is to utilize an LBP (low density lipoprotein binding protein) gene and/or polypeptide, or fragments, analogs and variants thereof, to aid in the treatment, diagnosis and/or identification of therapeutic agents for atherosclerosis.

In one aspect, the invention features an isolated polynucleotide comprising a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9; or a polynucleotide capable of hybridizing to and which is at least about 95% identical to any of the above polynucleotides and wherein the encoded polypeptide is capable of binding to LDL; or a biologically active fragment of any of the above polynucleotides wherein the encoded polypeptide is capable of binding to LDL.

In certain embodiments, the polynucleotide comprises the nucleic acid sequence as set forth in SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 or SEQ ID NO:18.

Another aspect of the invention is an isolated polypeptide comprising a polypeptide having the amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9; or a polypeptide which is at least about 95% identical to any of the above polypeptides and wherein the polypeptide is capable of binding to LDL; or a biologically active fragment of any of the above polypeptides wherein the fragment is capable of binding to LDL.

Another aspect of the invention is a method for determining if an animal is at risk for atherosclerosis. An animal is provided. An aspect of LBP metabolism or structure is evaluated in the animal. An abnormality in the aspect of LBP metabolism or structure is diagnostic of being at risk for atherosclerosis.

Another aspect of the invention is a method for evaluating an agent for use in treating atherosclerosis. A test cell, cell-free system or animal is provided. An agent is provided. The agent is administered to the test cell, cell-free system or animal in a therapeutically effective amount. The effect of the agent on an aspect of LBP metabolism or structure is evaluated. A change in the aspect of LBP metabolism or structure is indicative of the usefulness of the agent

in treating atherosclerosis.

Another aspect of the invention is a method for evaluating an agent for the ability to alter the binding of LBP polypeptide to a binding molecule, e.g., nat.ve LDL, modified LDL, e.g., methylated LDL or oxidized LDL, or an arterial extracellular matrix structural component. An agent is provided. An LBP polypeptide is provided. A binding molecule is provided. The agent, LBP polypeptide and binding molecule are combined. The formation of a complex comprising the LBP polypeptide and binding molecule is detected. An alteration in the formation of the complex in the presence of the agent as compared to in the absence of the agent is indicative of the agent altering the binding of the LBP polypeptide to the binding molecule.

Another aspect of the invention is a method for evaluating an agent for the ability to bind to an LBP polypeptide. An agent is provided. An LBP polypeptide is provided. The agent is contacted with the LBP polypeptide. The ability of the agent to bind to the LBP polypeptide is evaluated.

Another aspect of the invention is a method for evaluating an agent for the ability to bind to a nucleic acid encoding an LBP regulatory sequence. An agent is provided. A nucleic acid encoding an LBP regulatory sequence is provided. The agent is contacted with the nucleic acid. The ability of the agent to bind to the nucleic acid is evaluated.

Another aspect of the invention is a method for treating atherosclerosis in an animal. An animal in need of treatment for atherosclerosis is provided. An agent capable of altering an aspect of LBP structure or metabolism is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the atherosclerosis occurs. In certain embodiments, the agent is an LBP polypeptide, e.g., LBP-1, LBP-2 or LBP-3, or a biologically active fragment or analog thereof. In certain embodiments, the agent is a polypeptide of no more than about 100, 50, 30, 20, 10, 5, 4, 3 or 2 amino acid residues in length. In certain embodiments, the agent is a polypeptide having an amino acid sequence that includes at least about 20%, 40%, 60%, 80%, 90%, 95% or 98% acidic amino acid residues.

Another aspect of the invention is a method for treating an animal at risk for atherosclerosis. An animal at risk for atherosclerosis is provided. An agent capable of altering an aspect of LBP structure or metabolism is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the animal occurs.

Another aspect of the invention is a method for treating a cell having an abnormality in structure or metabolism of LBP. A cell having an abnormality in structure or metabolism of

LBP is provided. An agent capable of altering an aspect of LBP structure or metabolism is provided. The agent is administered to the cell in a therapeutically effective amount such that treatment of the cell occurs.

Another aspect of the invention is a pharmaceutical composition for treating atherosclerosis in an animal comprising a therapeutically effective amount of an agent, the agent being capable of altering an aspect of LBP metabolism or structure in the animal so as to result in treatment of the atherosclerosis, and a pharmaceutically acceptable carrier.

Another aspect of the invention is a vaccine composition for treating atherosclerosis in an animal comprising a therapeutically effective amount of an agent, the agent being capable of altering an aspect of LBP metabolism or structure in the animal so as to result in treatment of the atherosclerosis, and a pharmaceutically acceptable carrier.

Another aspect of the invention is a method for diagnosing atherosclerotic lesions in an animal. An animal is provided. A labeled agent capable of binding to LBP, e.g., LBP-1, LBP-2 or LBP-3, present in atherosclerotic lesions is provided. The labeled agent is administered to the animal under conditions which allow the labeled agent to interact with the LBP so as to result in labeled LBP. The localization or quantification of the labeled LBP is determined by imaging so as to diagnose the presence of atherosclerotic lesions in the animal.

Another aspect of the invention is a method for immunizing an animal against an LBP, e.g., LBP-1, LBP-2 or LBP-3, or fragment or analog thereof. An animal having LDL is provided. The LBP or fragment or analog thereof is administered to the animal so as to stimulate antibody production by the animal to the LBP or fragment or analog thereof such that binding of the LBP to the LDL is altered, e.g., decreased or increased.

Another aspect of the invention is a method of making a fragment or analog of LBP polypeptide, the fragment or analog having the ability to bind to native LDL and to modified LDL, e.g., methylated LDL, oxidized LDL, acetylated LDL, or cyclohexanedione-treated LDL. An LBP polypeptide is provided. The sequence of the LBP polypeptide is altered. The altered LBP polypeptide is tested for the ability to bind to modified LDL and native LDL.

Yet another aspect of the invention is a method for isolating a cDNA encoding an LBP. A cDNA library is provided. The cDNA library is screened for a cDNA encoding a polypeptide which binds to native LDL and modified LDL, e.g., methylated LDL or oxidized LDL. The cDNA which encodes the polypeptide is isolated, the cDNA encoding an LBP.

The above and other features, objects and advantages of the present invention will be

better understood by a reading of the following specification in conjunction with the drawings.

Brief Description of the Drawings

Fig. 1 depicts the amino acid sequence of rabbit LBP-1 (SEQ ID NC:1). Differences in amino acids between rabbit and human LBP-1 are depicted in bold type.

Fig. 2 depicts the amino acid sequence of rabbit LBP-2 (SEQ ID NO:2). Differences in amino acids between rabbit and human LBP-2 are depicted in bold type.

Fig. 3 depicts the amino acid sequence of amino acids 86 to 317 of rabbit LBP-2 (SEQ ID NO:3).

Fig. 4 depicts the amino acid sequence of amino acids 66 to 317 of rabbit LBP-2 (SEQ ID NO:4).

Fig. 5 depicts the amino acid sequence of rabbit LBP-3 (SEQ ID NO:5). Differences in amino acids between rabbit and human LBP-3 are depicted in bold type.

Fig. 6 depicts the amino acid sequence of human LBP-1 (SEQ ID NO:6). Differences in amino acids between rabbit and human LBP-1 are depicted in bold type.

Fig. 7 depicts the amino acid sequence of human LBP-2 (SEQ ID NO:7). Differences in amino acids between rabbit and human LBP-2 are depicted in bold type.

Fig. 8 depicts the amino acid sequence of human LBP-3 (SEQ ID NO:8). Differences in amino acids between rabbit and human LBP-3 are depicted in bold type.

Fig. 9 depicts the amino acid sequence of amino acids 14 to 33 of human or rabbit LBP-1, called BHF-1 (SEQ ID NO:9).

Fig. 10 depicts the cDNA sequence encoding rabbit LBP-1 (SEQ ID NO:10) and the corresponding amino acid sequence. Differences in amino acids between rabbit and human LBP-1 are depicted in bold type.

Fig. 11 depicts the cDNA sequence encoding rabbit LBP-2 (SEQ ID NO:11) and the corresponding amino acid sequence. Differences in amino acids between rabbit and human LBP-2 are depicted in bold type.

Fig. 12 depicts the cDNA sequence 256 to 1617 of rabbit LBP-2 (SEQ ID NO:12) and the corresponding amino acid sequence.

Fig. 13 depicts the cDNA sequence 196 to 1617 of rabbit LBP-2 (SEQ ID NO:13) and the corresponding amino acid sequence.

Fig. 14 depicts the cDNA sequence encoding rabbit LBP-3 (SEQ ID NO:14) and the corresponding amino acid sequence. Differences in amino acids between rabbit and human

LBP-3 are depicted in bold type.

Fig. 15 depicts the cDNA sequence encoding human LBP-1 (SEQ ID NO:15) and the corresponding amino acid sequence. Differences in amino acids between rabbit and human LBP-1 are depicted in bold type.

Fig. 16 depicts the cDNA sequence encoding human LBP-2 (SEQ ID NO:16) and the corresponding amino acid sequence. Differences in amino acids between rabbit and human LBP-2 are depicted in bold type.

Fig. 17 depicts the cDNA sequence encoding human LBP-3 (SEQ ID NO:17) and the corresponding amino acid sequence. Differences in amino acids between rabbit and human LBP-3 are depicted in bold type.

Fig. 18 depicts the cDNA sequence encoding BHF-1 (SEQ ID NO:18).

Fig. 19 corresponds to the amino acid sequence of rabbit LBP-1 (top sequence) in alignment with the amino acid sequence of human LBP-1 (bottom sequence).

Fig. 20 corresponds to the amino acid sequence of rabbit LBP-2 (top sequence) in alignment with the amino acid sequence of human LBP-2 (bottom sequence).

Fig. 21 corresponds to the amino acid sequence of rabbit LBP-3 (top sequence) in alignment with the amino acid sequence of human LBP-3 (bottom sequence).

Detailed Description

In accordance with aspects of the present invention, there are provided novel mature human and rabbit polypeptides, LBP-1, LBP-2 and LBP-3, and biologically active analogs and fragments thereof, and there are provided isolated polynucleotides which encode such polypeptides. LBP is an abbreviation for low density lipoprotein (LDL) binding protein. The terms polynucleotide, nucleotide and oligonucleotide are used interchangeably herein, and the terms polypeptides, proteins and peptides are used interchangeably herein.

This invention provides for an isolated polynucleotide comprising a polynucleotide encoding the polypeptide having the amino acid sequence of rabbit LBP-1 as set forth in Fig. 1 (SEQ ID NO:1); rabbit LBP-2 as set forth in Fig. 2 (SEQ ID NO:2); 86 to 317 of rabbit LBP-2 as set forth in Fig. 3 (SEQ ID NO:3); 66 to 317 of rabbit LBP-2 as set forth in Fig. 4 (SEQ ID NO:4); rabbit LBP-3 as set forth in Fig. 5 (SEQ ID NO:5); human LBP-1 as set forth in Fig. 6 (SEQ ID NO:6); human LBP-2 as set forth in Fig. 7 (SEQ ID NO:7); human LBP-3 as set forth in Fig. 8 (SEQ ID NO:8); 14 to 33 of human or rabbit LBP-1, called BHF-1, as set forth in Fig. 9 (SEQ ID NO:9); a polynucleotide capable of hybridizing to and which is at least about 80%

identical, more preferably at least about 90% identical, more preferably yet at least about 95% identical, and most preferably at least about 98% identical to any of the above polynucleotides, and wherein the encoded polypeptide is capable of binding to LDL; or a biologically active fragment of any of the above polynucleotides wherein the encoded polypeptide is capable of binding to LDL.

This invention also includes an isolated polynucleotide comprising a polynucleotide encoding the polypeptide having amino acid residues 8-22 (SEQ ID NO:19), 8-33 (SEQ ID NO:20), 23-33 (SEQ ID NO:21) or 208-217 (SEQ ID NO:22) of human LBP-2 as set forth in Fig. 7 (SEQ ID NO:7); amino acid residues 14-43 (SEQ ID NO:23) or 38-43 (SEQ ID NO:24) of rabbit or human LBP-1 as set forth in Fig. 1 (SEQ ID NO:1) and Fig. 6 (SEQ ID NO:6); amino acid residues 105-120 (SEQ ID NO:25), 105-132 (SEQ ID NO:26), 121-132 (SEQ ID NO:27) or 211-220 (SEQ ID NO:28) of rabbit LBP-2 as set forth in Fig. 2 (SEQ ID NO:2); amino acid residues 96-110 (SEQ ID NO:29) of rabbit LBP-3 as set forth in Fig. 5 (SEQ ID NO:5); amino acid residues 53-59 (SEQ ID NO:41) of human LBP-3 as set forth in Fig. 8 (SEQ ID NO:8); a polynucleotide capable of hybridizing to and which is at least about 80% identical, more preferably at least about 90% identical, more preferably yet at least about 95% identical, and most preferably at least about 98% identical to any of the above polynucleotides, and wherein the encoded polypeptide is capable of binding to LDL; or a biologically active fragment of any of the above polynucleotides wherein the encoded polypeptide is capable of binding to LDL.

By a polynucleotide encoding a polypeptide is meant a polynucleotide which includes only coding sequence for the polypeptide, as well as a polynucleotide which includes additional coding and/or non-coding sequences. Thus, e.g., the polynucleotides which encode for the mature polypeptides of Figs. 1-9 (SEQ ID NOS:1-9) may include only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequences 5' and/or 3' of the coding sequence for the mature polypeptide. The polynucleotides of the invention are also meant to include polynucleotides in which the coding sequence for the mature polypeptide is fused in the same reading frame to a polynucleotide sequence which aids in expression and/or secretion of a polypeptide from a host cell, e.g., a leader sequence. The polynucleotides are also meant to include polynucleotides in which the coding sequence is fused in frame to a marker sequence

which, e.g., allows for purification of the polypeptide.

The polynucleotides of the present invention may be in the form of RNA, DNA or PNA, e.g., cRNA, cDNA, genomic DNA, or synthetic DNA, RNA or PNA. The DNA may be double-stranded or single stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand.

In preferred embodiments, the polynucleotide comprises the nucleic acid of rabbit LBP-1 as set forth in Fig. 10 (SEQ ID NO:10); rabbit LBP-2 as set forth in Fig. 11 (SEQ ID NO:11); nucleotide 256 to 1617 of rabbit LBP-2 as set forth in Fig. 12 (SEQ ID NO:12); nucleotide 196 to 1617 of rabbit LBP-2 as set forth in Fig. 13 (SEQ ID NO:13); rabbit LBP-3 as set forth in Fig. 14 (SEQ ID NO:14); human LBP-1 as set forth in Fig. 15 (SEQ ID NO:15); human LBP-2 as set forth in Fig. 16 (SEQ ID NO:16); human LBP-3 as set forth in Fig. 17 (SEQ ID NO:17); or nucleotide 97 to 156 of rabbit LBP-1 or nucleotide 157 to 216 of human LBP-1, (BHF-1), as set forth in Fig. 18 (SEQ ID NO:18).

In other preferred embodiments, the polynucleotide comprises the nucleic acid as set forth in SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40 or SEQ ID NO:42.

The coding sequence which encodes the mature polypeptide may be identical to the coding sequences shown in Figs. 10-18 (SEQ ID NOS:10-18) or SEQ ID NOS:30-40 or 42, or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptides as the DNA of Figs. 10-18 (SEQ ID NOS:10-18) and SEQ ID NOS: 30-40 and 42.

This invention also includes recombinant vectors comprising the polynucleotides described above. The vector can be, e.g., a plasmid, a viral particle or a phage. In certain embodiments, the recombinant vector is an expression vector. The vectors may also include various marker genes which are useful in identifying cells containing such vectors.

This invention also includes a cell comprising such a recombinant vector. The recombinant vectors described herein can be introduced into a host cell, e.g., by transformation, transfection or infection.

This invention also includes a method for producing an LBP comprising culturing such a cell under conditions that permit expression of the LBP.

This invention also includes an isolated polypeptide comprising a polypeptide having the

amino acid sequence as set forth in Fig. 1 (SEQ ID NO:1); Fig. 2 (SEQ ID NO:2); Fig. 3 (SEQ ID NO:3); Fig. 4 (SEQ ID NO:4); Fig. 5 (SEQ ID NO:5); Fig. 6 (SEQ ID NO:6); Fig. 7 (SEQ ID NO:7); Fig. 8 (SEQ ID NO:8) or Fig. 9 (SEQ ID NO:9); or a polypeptide which is at least about 80% identical, more preferably at least about 90% identical, more preferably yet at least about 95% identical, and most preferably at least about 98% identical to the above polypeptides, and wherein said polypeptide is capable of binding to LDL; or a biologically active fragment of any of the above polypeptides wherein the fragment is capable of binding to LDL. Differences in amino acids between the rabbit and human LBP-1, LBP-2 and LBP-3 genes are depicted in bold type in the figures. The differences in the amino acid sequences between rabbit and human LBP-1, LBP-2 and LBP-3 are also specifically shown in Figs. 19, 20 and 21, respectively.

This invention also includes an isolated polypeptide comprising a polypeptide having amino acid residues 8-22 (SEQ ID NO:19), 8-33 (SEQ ID NO:20), 23-33 (SEQ ID NO:21) or 208-217 (SEQ ID NO:22) as set forth in Fig. 7 (SEQ ID NO:7); amino acid residues 14-43 (SEQ ID NO:23) or 38-43 (SEQ ID NO:24) as set forth in Fig. 1 (SEQ ID NO:1) and Fig. 6 (SEQ ID NO:6); amino acid residues 105-120 (SEQ ID NO:25), 105-132 (SEQ ID NO:26), 121-132 (SEQ ID NO:27) or 211-220 (SEQ ID NO:28) as set forth in Fig. 2 (SEQ ID NO:2); amino acid residues 96-110 (SEQ ID NO:29) as set forth in Fig. 5 (SEQ ID NO:5); and amino acid residues 53-59 (SEQ ID NO:41) as set forth in Fig. 8 (SEQ ID NO:8); or a polypeptide which is at least about 80% identical, more preferably at least about 90% identical, more preferably yet at least about 95% identical, and most preferably at least about 98% identical to the above polypeptides, and wherein said polypeptide is capable of binding to LDL; or a biologically active fragment of any of the above polypeptides wherein the fragment is capable of binding to LDL.

The polypeptides of the invention are meant to include, e.g., a naturally purified product, a chemically synthesized product, and a recombinantly derived product.

The polypeptides can be used, e.g., to bind to LDL, thereby inhibiting formation of atherosclerotic plaques. The polypeptides can also be used, e.g., in gene therapy, by expression of such polypeptides in vivo. The polypeptides can also be used in pharmaceutical or vaccine compositions. The polypeptides can also be used as immunogens to produce antibodies thereto, which in turn, can be used as antagonists to the LBP polypeptides.

Without being bound by any theory, it is believed that the LBPs provide the mechanism by which atherosclerosis is promoted through LDL oxidation. The LBPs are believed to be required in order for focal, irreversible LDL binding to occur at the arterial wall, and that such

binding is a critical early event in atherosclerosis because it allows the time necessary for LDL to be changed from its native state to a fully oxidized state. Since oxidized, but not native, LDL is a foreign protein, macrophages ingest it, first becoming the foam cells of type I lesions, and subsequently forming the fatty streaks of type II lesions.

This invention also includes a method for determining if an animal is at risk for atherosclerosis. An animal is provided. An aspect of LBP metabolism or structure is evaluated in the animal. An abnormality in the aspect of LBP metabolism or structure is diagnostic of being at risk for atherosclerosis.

By atherosclerosis is meant a disease or condition which comprises several stages which blend imperceptibly into each other, including irreversible binding of LDL, LDL oxidation, macrophage recruitment, blockage of the artery and tissue death (infarction).

By animal is meant human as well as non-human animals. Non-human animals include, e.g., mammals, birds, reptiles, amphibians, fish, insects and protozoa. Preferably, the non-human animal is a mammal, e.g., a rabbit, a rodent, e.g., a mouse, rat or guinea pig, a primate, e.g., a monkey, or a pig. An animal also includes transgenic non-human animals. The term transgenic animal is meant to include an animal that has gained new genetic information from the introduction of foreign DNA, i.e., partly or entirely heterologous DNA, into the DNA of its cells; or introduction of a lesion, e.g., an in vitro induced mutation, e.g., a deletion or other chromosomal rearrangement into the DNA of its cells; or introduction of homologous DNA into the DNA of its cells in such a way as to alter the genome of the cell into which the DNA is inserted, e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout or replacement of the homologous host gene or results in altered and/or regulatable expression and/or metabolism of the gene. The animal may include a transgene in all of its cells including germ line cells, or in only one or some of its cells. Transgenic animals of the invention can serve as a model for studying atherosclerosis or for evaluating agents to treat atherosclerosis.

In certain embodiments, the determination for being at risk for atherosclerosis is done in a prenatal animal.

By LBP is meant a low density lipoprotein (LDL) binding protein which is capable of binding LDL and methylated LDL. By methylated LDL is meant that about 50% to about 90% of the lysine residues of LDL have a methyl group chemically attached. Methylated LDL is not recognized by previously reported cell surface receptors. See, e.g., Weisgraber et al., J. Biol.

Chem. 253:9053-9062 (1978). In certain embodiments, the LBP is also capable of binding oxidized LDL. In certain preferred embodiments, the binding of LDL to an LBP is irreversible. In certain preferred embodiments, the LBP does not transport the LDL to any intracellular compartment. Examples of LBPs are LBP-1, LBP-2 and LBP-3 described herein.

By LBP metabolism is meant any aspect of the production, release, expression, function, action, interaction or regulation of LBP. The metabolism of LBP includes modifications, e.g., covalent or non-covalent modifications, of LBP polypeptide. The metabolism of LBP includes modifications, e.g., covalent or non-covalent modifications, that LBP induces in other substances. The metabolism of LBP also includes changes in the distribution of LBP polypeptide, and changes LBP induces in the distribution of other substances.

Any aspect of LBP metabolism can be evaluated. The methods used are standard techniques known to those skilled in the art and can be found in standard references, e.g., Ausubel et al., ed., Current Protocols in Mol. Biology, New York: John Wiley & Sons, 1990; Kriegler, M., ed., Gene Transfer and Expression, Stockton Press, New York, NY, 1989; pDisplay gene expression system (Invitrogen, Carlsbad, CA). Preferred examples of LBP metabolism that can be evaluated include the binding activity of LBP polypeptide to a binding molecule, e.g., LDL; the transactivation activity of LBP polypeptide on a target gene; the level of LBP protein; the level of LBP mRNA; the level of LBP modifications, e.g., phosphorylation, glycosylation or acylation; or the effect of LBP expression on transfected mammalian cell binding of LDL.

By binding molecule is meant any molecule to which LBP can bind, e.g., a nucleic acid, e.g., a DNA regulatory region, a protein, e.g., LDL, a metabolite, a peptide mimetic, a non-peptide mimetic, an antibody, or any other type of ligand. In certain preferred embodiments, the aspect of LBP metabolism that is evaluated is the ability of LBP to bind to native LDL and/or methylated LDL and/or oxidized LDL. Binding to LDL can be shown, e.g., by antibodies against LDL, affinity chromatography, affinity coelectrophoresis (ACE) assays, or ELISA assays. See Examples. In other embodiments, it is the ability of LBP to bind to an arterial extracellular matrix stuctural component that is evaluated. Examples of such components include proteoglycans, e.g., chondroitin sulfate proteoglycans and heparin sulfate proteoglycans; elastin; collagen; fibronectin; vitronectin; integrins; and related extracellular matrix molecules. Binding to arterial extracellular matrix structural components can be shown by standard methods known to those skilled in the art, e.g., by ELISA assays. Primary antibodies to the LBP are then added, followed by an enzyme-conjugated secondary antibody to the primary antibody, which

produces a stable color in the presence of an appropriate substrate, and color development on the plates is measured in a microtiter plate reader.

Transactivation of a target gene by LBP can be determined, e.g., in a transient transfection assay in which the promoter of the target gene is linked to a reporter gene, e.g., β-galacto-sidase or luciferase, and co-transfected with an LBP expression vector. Such evaluations can be done in vitro or in vivo. Levels of LBP protein, mRNA or phosphorylation, can be measured, e.g., in a sample, e.g., a tissue sample, e.g., arterial wall, by standard methods known to those skilled in the art.

In certain embodiments, an aspect of LBP structure is evaluated, e.g., LBP gene structure or LBP protein structure. For example, primary, secondary or tertiary structures can be evaluated. For example, the DNA sequence of the gene is determined and/or the amino acid sequence of the protein is determined. Standard cloning and sequencing methods can be used as are known to those skilled in the art. In certain embodiments, the binding activity of an antisense nucleic acid with the cellular LBP mRNA and/or genomic DNA is determined using standard methods known to those skilled in the art so as to detect the presence or absence of the target mRNA or DNA sequences to which the antisense nucleic acid would normally specifically bind.

The risk for atherosclerosis that is determined can be a reduced risk or an increased risk as compared to a normal animal. For example, an abnormality which would give a reduced risk is an inactive LBP polypeptide. An abnormality which would give an increased risk would be, e.g., an LBP polypeptide that has higher activity, e.g., LDL binding activity, than native LBP polypeptide.

The invention also includes a method for evaluating an agent for use in treating atherosclerosis. A test cell, cell-free system or animal is provided. An agent is provided. The agent is administered to the test cell, cell-free system or animal in a therapeutically effective amount. The effect of the agent on an aspect of LBP metabolism or structure is evaluated. A change in the aspect of LBP metabolism or structure is indicative of the usefulness of the agent in treating atherosclerosis.

In certain embodiments, the method employs two phases for evaluating an agent for use in treating atherosclerosis, an initial in vitro phase and then an in vivo phase. The agent is administered to the test cell or cell-free system in vitro, and if a change in an aspect of LBP metabolism occurs, then the agent is further administered to a test animal in a therapeutically effective amount and evaluated in vivo for an effect of the agent on an aspect of LBP

metabolism.

By cell is meant a cell or a group of cells, or a cell that is part of an animal. The cell can be a human or non-human cell. Cell is also meant to include a transgenic cell. The cell can be obtained, e.g., from a culture or from an animal. Animals are meant to include, e.g., natural animals and non-human transgenic animals. In certain embodiments, the transgenic cell or non-human transgenic animal has an LBP transgene, or fragment or analog thereof. In certain embodiments, the transgenic cell or non-human transgenic animal has a knockout for the LBP gene.

The test cell, cell-free system or animal can have a wild type pattern or a non-wild type pattern of LBP metabolism. A non-wild type pattern of LBP metabolism can result, e.g., from under-expression, over-expression, no expression, or a temporal, site or distribution change. Such a non-wild type pattern can result, e.g., from one or more mutations in the LBP gene, in a binding molecule gene, a regulatory gene, or in any other gene which directly or indirectly affects LBP metabolism. A mutation is meant to include, e.g., an alteration, e.g., in gross or fine structure, in a nucleic acid. Examples include single base pair alterations, e.g., missense or nonsense mutations, frameshifts, deletions, insertions and translocations. Mutations can be dominant or recessive. Mutations can be homozygous or heterozygous. Preferably, an aspect of LBP-1, LBP-2 or LBP-3 metabolism is evaluated.

An agent is meant to include, e.g., any substance, e.g., an anti-atherosclerosis drug. The agent of this invention preferably can change an aspect of LBP metabolism. Such change can be the result of any of a variety of events, including, e.g., preventing or reducing interaction between LBP and a binding molecule, e.g., LDL or an arterial extracellular matrix structural component; inactivating LBP and/or the binding molecule, e.g., by cleavage or other modification; altering the affinity of LBP and the binding molecule for each other; diluting out LBP and/or the binding molecule; preventing expression of LBP and/or the binding molecule; reducing synthesis of LBP and/or the binding molecule; synthesizing an abnormal LBP and/or binding molecule; synthesizing an alternatively spliced LBP and/or binding molecule; preventing or reducing proper conformational folding of LBP and/or the binding molecule; modulating the binding properties of LBP and/or the binding molecule; interfering with signals that are required to activate or deactivate LBP and/or the binding molecule; activating or deactivating LBP and/or the binding molecule in such a way as to prevent binding; or interfering with other receptors, ligands or other molecules which are required for the normal synthesis or functioning of LBP

and/or the binding molecule. For example, the agent can block the binding site on LDL for LBPs expressed focally in the arterial wall extracellular matrix, or it could block the binding site on an LBP for LDL, or it could be bifunctional, i.e., it could block both binding sites.

Examples of agents include LBP polypeptide, e.g., LBP-1, LBP-2 or LBP-3, or a biologically active fragment or analog thereof; a nucleic acid encoding LBP polypeptide or a biologically active fragment or analog thereof; a nucleic acid encoding an LBP regulatory sequence or a biologically active fragment or analog thereof; a binding molecule for LBP polypeptide; a binding molecule for LBP nucleic acid, the LBP nucleic acid being, e.g., a nucleic acid comprising a regulatory region for LBP or a nucleic acid comprising a structural region for LBP or a biologically active fragment of LBP; an antisense nucleic acid; a mimetic of LBP or a binding molecule; an antibody for LBP or a binding molecule; a metabolite; or an inhibitory carbohydrate or glycoprotein. In certain embodiments, the agent is an antagonist, agonist or super agonist.

Knowledge of the existence of the sequence of the LBPs allows a search for natural or artificial ligands to regulate LDL levels in the treatment of atherosclerosis. In certain embodiments, the agent is a natural ligand for LBP. In certain embodiments, the agent is an artificial ligand for LBP.

By analog is meant a compound that differs from naturally occurring LBP in amino acid sequence or in ways that do not involve sequence, or both. Analogs of the invention generally exhibit at least about 80% homology, preferably at least about 90% homology, more preferably yet at least about 95% homology, and most preferably at least about 98% homology, with substantially the entire sequence of a naturally occurring LBP sequence, preferably with a segment of about 100 amino acid residues, more preferably with a segment of about 50 amino acid residues, more preferably yet with a segment of about 30 amino acid residues, more preferably yet with a segment of about 20 amino acid residues, more preferably yet with a segment of about 10 amino acid residues, more preferably yet with a segment of about 5 amino acid residues, more preferably yet with a segment of about 4 amino acid residues, more preferably yet with a segment of about 3 amino acid residues, and most preferably with a segment of about 2 amino acid residues. Non-sequence modifications include, e.g., in vivo or in vitro chemical derivatizations of LBP. Non-sequence modifications include, e.g., changes in phosphorylation, acetylation, methylation, carboxylation, or glycosylation. Methods for making such modifications are known to those skilled in the art. For example, phosphorylation can be

modified by exposing LBP to phosphorylation-altering enzymes, e.g., kinases or phosphatases.

Preferred analogs include LBP or biologically active fragments thereof whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish LBP biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other examples of conservative substitutions are shown in Table 1.

Table 1

CONSERVATIVE AMINO ACID SUBSTITUTIONS

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn, L-NMMA, L-NAME
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, β-Ala Acp
Histidine	H	D-His
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tryptophan	W	D-Trp, Phe, D-Phe, Tyr, D-Tyr
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Amino acid sequence variants of a protein can be prepared by any of a variety of methods known to those skilled in the art. For example, random mutagenesis of DNA which encodes a protein or a particular domain or region of a protein can be used, e.g., PCR mutagenesis (using, e.g., reduced *Taq* polymerase fidelity to introduce random mutations into a cloned fragment of DNA; Leung et al., BioTechnique 1:11-15 (1989)), or saturation mutagenesis (by, e.g., chemical treatment or irradiation of single-stranded DNA in vitro, and synthesis of a complementary DNA strand; Mayers et al., Science 229:242 (1985)). Random mutagenesis can also be accomplished by, e.g., degenerate oligonucleotide generation (using, e.g., an automatic DNA synthesizer to chemically synthesize degenerate sequences; Narang, Tetrahedron 39:3 (1983); Itakura et al., Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. A.G. Walton, Amsterdam: Elsevier, pp. 273-289 (1981)). Non-random or directed mutagenesis can be used to provide specific sequences or mutations in specific regions. These techniques can be used to create variants which include, e.g., deletions, insertions, or substitutions, of residues of the known amino acid sequence of a protein. The sites for mutation can be modified individually or in series, e.g., by (i) substituting first with conserved amino acids and then with more radical choices depending upon results achieved, (ii) deleting the target residue, (iii) inserting residues of the same or a different class adjacent to the located site, or (iv) combinations of the above. For example, analogs can be made by in vitro DNA sequence modifications of the sequences of Figs. 10-18 (SEQ ID NOS:10-18). For example, in vitro mutagenesis can be used to convert any of these DNA sequences into a sequence which encodes an analog in which one or more amino acid residues has undergone a replacement, e.g., a conservative replacement as described in Table 1.

Methods for identifying desirable mutations include, e.g., alanine scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)), oligonucleotide-mediated mutagenesis (Adelman et al., DNA 2:183 (1983)); cassette mutagenesis (Wells et al., Gene 34:315 (1985)), combinatorial mutagenesis, and phage display libraries (Ladner et al., PCT International Appln. No. WO88/06630). The LBP analogs can be tested, e.g., for their ability to bind to LDL and/or to an arterial extracellular matrix component, as described herein.

Other analogs within the invention include, e.g., those with modifications which increase peptide stability. Such analogs may contain, e.g., one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are, e.g.: analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids; and cyclic analogs.

Analogs are also meant to include peptides in which structural modifications have been introduced into the peptide backbone so as to make the peptide non-hydrolyzable. Such peptides are particularly useful for oral administration, as they are not digested. Peptide backbone modifications include, e.g., modifications of the amide nitrogen, the α-carbon, the amide carbonyl, or the amide bond, and modifications involving extensions, deletions or backbone crosslinks. For example, the backbone can be modified by substitution of a sulfoxide for the carbonyl, by reversing the peptide bond, or by substituting a methylene for the carbonyl group. Such modifications can be made by standard procedures known to those skilled in the art. See, e.g., Spatola, A.F., "Peptide Backbone Modifications: A Structure-Activity Analysis of Peptides Containing Amide Bond Surrogates, Conformational Constraints, and Related Backbone Replacements," in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp. 267-357, B. Weinstein (ed.), Marcel Dekker, Inc., New York (1983).

An analog is also meant to include polypeptides in which one or more of the amino acid residues include a substituent group, or polypeptides which are fused with another compound, e.g., a compound to increase the half-life of the polypeptide, e.g., polyethylene glycol.

By fragment is meant some portion of the naturally occurring LBP polypeptide. Preferably, the fragment is at least about 100 amino acid residues, more preferably at least about 50 amino acid residues, more preferably yet at least about 30 amino acid residues, more preferably yet at least about 20 amino acid residues, more preferably yet at least about 5 amino acid residues, more preferably yet at least about 4 amino acid residues, more preferably yet at least about 3 amino acid residues, and most preferably at least about 2 amino acid residues in length. Fragments include, e.g., truncated secreted forms, proteolytic fragments, splicing fragments, other fragments, and chimeric constructs between at least a portion of the relevant gene, e.g., LBP-1, LBP-2 or LBP-3, and another molecule. Fragments of LBP can be generated by methods known to those skilled in the art. In certain embodiments, the fragment is biologically active. The ability of a candidate fragment to exhibit a biological activity of LBP can be assessed by methods known to those skilled in the art. For example, LBP fragments can be tested for their ability to bind to LDL and/or to an arterial extracellular matrix structural component, as described herein. Also included are LBP fragments containing residues that are not required for biological activity of the fragment or that result from alternative mRNA splicing or alternative protein processing events.

Fragments of a protein can be produced by any of a variety of methods known to those

skilled in the art, e.g., recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments. Digestion with "end-nibbling" endonucleases can thus generate DNAs which encode an array of fragments. DNAs which encode fragments of a protein can also be generated, e.g., by random shearing, restriction digestion or a combination of the above-discussed methods. For example, fragments of LBP can be made by expressing LBP DNA which has been manipulated in vitro to encode the desired fragment, e.g., by restriction digestion of any of the DNA sequences of Figs. 10-18 (SEQ ID NOS:10-18).

Fragments can also be chemically synthesized using techniques known in the art, e.g., conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, peptides of the present invention can be arbitrarily divided into fragments of desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length.

An LBP or a biologically active fragment or analog thereof, or a binding molecule or a biologically active fragment or analog thereof, can, e.g., compete with its cognate molecule for the binding site on the complementary molecule, and thereby reduce or eliminate binding between LBP and the cellular binding molecule. LBP or a binding molecule can be obtained, e.g., from purification or secretion of naturally occurring LBP or binding molecule, from recombinant LBP or binding molecule, or from synthesized LBP or binding molecule.

Therefore, methods for generating analogs and fragments and testing them for activity are known to those skilled in the art.

An agent can also be a nucleic acid used as an antisense molecule. Antisense therapy is meant to include, e.g., administration or in situ generation of oligonucleotides or their derivatives which specifically hybridize, e.g., bind, under cellular conditions, with the cellular mRNA and/or genomic DNA encoding an LBP polypeptide, or mutant thereof, so as to inhibit expression of the encoded protein, e.g., by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix.

In certain embodiments, the antisense construct binds to a naturally-occurring sequence of an LBP gene which, e.g., is involved in expression of the gene. These sequences include, e.g., promoter, start codons, stop codons, and RNA polymerase binding sites.

In other embodiments, the antisense construct binds to a nucleotide sequence which is not present in the wild type gene. For example, the antisense construct can bind to a region of an LBP gene which contains an insertion of an exogenous, non-wild type sequence. Alternatively, the antisense construct can bind to a region of an LBP gene which has undergone a deletion, thereby bringing two regions of the gene together which are not normally positioned together and which, together, create a non-wild type sequence. When administered in vivo to a subject, antisense constructs which bind to non-wild type sequences provide the advantage of inhibiting the expression of a mutant LBP gene, without inhibiting expression of any wild type LBP gene.

An antisense construct of the present invention can be delivered, e.g., as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA which encodes an LBP polypeptide. An alternative is that the antisense construct is an oligonucleotide which is generated ex vivo and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA (duplexing) and/or genomic sequences (triplexing) of an LBP gene. Such oligonucleotides are preferably modified oligonucleotides which are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, and are therefore stable in vivo. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate, phosphorodithioates and methylphosphonate analogs of DNA and peptide nucleic acids (PNA). (See also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed. (See, e.g., Van der Krol et al., Biotechniques 6:958-976, (1988); Stein et al., Cancer Res. 48:2659-2668 (1988)).

By mimetic is meant a molecule which resembles in shape and/or charge distribution LBP or a binding molecule. The mimetic can be a peptide or a non-peptide. Mimetics can act as therapeutic agents because they can, e.g., competitively inhibit binding of LBP to a binding molecule. By employing, e.g., scanning mutagenesis, e.g., alanine scanning mutagenesis, linker scanning mutagenesis or saturation mutagenesis, to map the amino acid residues of a particular LBP polypeptide involved in binding a binding molecule, peptide mimetics, e.g., diazepine or isoquinoline derivatives, can be generated which mimic those residues in binding to a binding molecule, and which therefore can inhibit binding of the LBP to a binding molecule and thereby interfere with the function of LBP. Non-hydrolyzable peptide analogs of such residues can be generated using, e.g., benzodiazepine (see, e.g., Freidinger et al., in Peptides: Chemistry and Biology, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands (1988)); azepine (see, e.g.,

Huffman et al., in Peptides: Chemistry and Biology, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands (1988)); substituted gamma lactam rings (see, e.g., Garvey et al., in Peptides: Chemistry and Biology, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands (1988)); keto-methylene pseudopeptides (see, e.g., Ewenson et al., J. Med. Chem. 29:295 (1986); Ewenson et al., in Peptides: Structure and Function (Proceedings of the 9th American Peptide Symposium) Pierce Chemical Co. Rockland, IL (1985)); β-turn dipeptide cores (see, e.g., Nagai et al., Tetrahedron Lett. 26:647 (1985); Sato et al., J. Chem. Soc. Perkin Trans. 1:1231 (1986)); or β-aminoalcohols (see, e.g., Gordon et al., Biochem. Biophys. Res. Commun. 126:419 (1985); Dann et al., Biochem. Biophys. Res. Commun. 134:71 (1986)).

Antibodies are meant to include antibodies against any moiety that directly or indirectly affects LBP metabolism. The antibodies can be directed against, e.g., LBP or a binding molecule, or a subunit or fragment thereof. For example, antibodies include anti-LBP-1, LBP-2 or LBP-3 antibodies; and anti-binding molecule antibodies. Antibody fragments are meant to include, e.g., Fab fragments, Fab' fragments, $F(ab')_2$ fragments, F(v) fragments, heavy chain monomers, heavy chain dimers, heavy chain trimers, light chain monomers, light chain dimers, light chain trimers, dimers consisting of one heavy and one light chain, and peptides that mimic the activity of the anti-LBP or anti-binding molecule antibodies. For example, Fab_2' fragments of the inhibitory antibody can be generated through, e.g., enzymatic cleavage. Both polyclonal and monoclonal antibodies can be used in this invention. Preferably, monoclonal antibodies are used. Natural antibodies, recombinant antibodies or chimeric-antibodies, e.g., humanized antibodies, are included in this invention. Preferably, humanized antibodies are used when the subject is a human. Most preferably, the antibodies have a constant region derived from a human antibody and a variable region derived from an inhibitory mouse monoclonal antibody. Production of polyclonal antibodies to LBP is described in Example 6. Monoclonal and humanized antibodies are generated by standard methods known to those skilled in the art. Monoclonal antibodies can be produced, e.g., by any technique which provides antibodies produced by continuous cell lines cultures. Examples include the hybridoma technique (Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today 4:72 (1983)), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., in Monoclonal Antibodies and Cancer Therapy, A.R. Liss, Inc., pp. 77-96 (1985)). Preferably, humanized antibodies are raised through conventional production and harvesting techniques (Berkower, I., Curr. Opin. Biotechnol. 7:622-

628 (1996); Ramharayan and Skaletsky, Am. Biotechnol. Lab 13:26-28 (1995)). In certain preferred embodiments, the antibodies are raised against the LBP, preferably the LDL-binding site, and the Fab fragments produced. These antibodies, or fragments derived therefrom, can be used, e.g., to block the LDL-binding sites on the LBP molecules.

Agents also include inhibitors of a molecule that are required for synthesis, post-translational modification, or functioning of LBP and/or a binding molecule, or activators of a molecule that inhibits the synthesis or functioning of LBP and/or the binding molecule. Agents include, e.g., cytokines, chemokines, growth factors, hormones, signaling components, kinases, phosphatases, homeobox proteins, transcription factors, editing factors, translation factors and post-translation factors or enzymes. Agents are also meant to include ionizing radiation, non-ionizing radiation, ultrasound and toxic agents which can, e.g., at least partially inactivate or destroy LBP and/or the binding molecule.

An agent is also meant to include an agent which is not entirely LBP specific. For example, an agent may alter other genes or proteins related to arterial plaque formation. Such overlapping specificity may provide additional therapeutic advantage.

The invention also includes the agent so identified as being useful in treating atherosclerosis.

The invention also includes a method for evaluating an agent for the ability to alter the binding of LBP polypeptide to a binding molecule. An agent is provided. An LBP polypeptide is provided. A binding molecule is provided. The agent, LBP polypeptide and binding molecule are combined. The formation of a complex comprising the LBP polypeptide and binding molecule is detected. An alteration in the formation of the complex in the presence of the agent as compared to in the absence of the agent is indicative of the agent altering the binding of the LBP polypeptide to the binding molecule.

In preferred embodiments, the LBP polypeptide is LBP-1, LBP-2 or LBP-3. Examples of a binding molecule include native LDL, modified LDL, e.g., methylated LDL or oxidized LDL, and arterial extracellular matrix structural components.

Altering the binding includes, e.g., inhibiting or promoting the binding. The efficacy of the agent can be assessed, e.g., by generating dose response curves from data obtained using various concentrations of the agent. Methods for determining formation of a complex are standard and are known to those skilled in the art, e.g., affinity coelectrophoresis (ACE) assays or ELISA assays as described herein.

The invention also includes the agent so identified as being able to alter the binding of an LBP polypeptide to a binding molecule.

The invention also includes a method for evaluating an agent for the ability to bind to an LBP polypeptide. An agent is provided. An LBP polypeptide is provided. The agent is contacted with the LBP polypeptide. The ability of the agent to bind to the LBP polypeptide is evaluated. Preferably, the LBP polypeptide is LBP-1, LBP-2 or LBP-3. Binding can be determined, e.g., by measuring formation of a complex by standard methods known to those skilled in the art, e.g., affinity coelectrophoresis (ACE) assays or ELISA assays as described herein.

The invention also includes the agent so identified as being able to bind to LBP polypeptide.

The invention also includes a method for evaluating an agent for the ability to bind to a nucleic acid encoding an LBP regulatory sequence. An agent is provided. A nucleic acid encoding an LBP regulatory sequence is provided. The agent is contacted with the nucleic acid. The ability of the agent to bind to the nucleic acid is evaluated. Preferably, the LBP regulatory sequence is an LBP-1, LBP-2 or LBP-3 regulatory sequence. Binding can be determined, e.g., by measuring formation of a complex by standard methods known to those skilled in the art, e.g., DNA mobility shift assays, DNase I footprint analysis (Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, (1989)).

The invention also includes the agent so identified as being able to bind to a nucleic acid encoding an LBP regulatory sequence.

The invention also includes a method for treating atherosclerosis in an animal. An animal in need of treatment for atherosclerosis is provided. An agent capable of altering an aspect of LBP structure or metabolism is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the atherosclerosis occurs.

In certain preferred embodiments, the agent is an LBP polypeptide, e.g., LBP-1, LBP-2 or LBP-3, or a biologically active fragment or analog thereof. The agent can be, e.g., the polypeptide as set forth in SEQ ID NOS:1-9. Preferably, the agent is a polypeptide of no more than about 100 amino acid residues in length, more preferably of no more than about 50 amino acid residues, more preferably yet of no more than about 30 amino acid residues, more preferably yet of no more than about 20 amino acid residues, more preferably yet of no more than about 10 amino acid residues, more preferably yet of no more than about 5 amino acid residues, more

preferably yet of no more than about 4 amino acid residues, more preferably yet of no more than about 3 amino acid residues, and most preferably of no more than about 2 amino acid residues. Preferably, the polypeptide includes at least about 20% acidic amino acid residues, more preferably yet at least about 40% acidic amino acid residues, more preferably yet at least about 60% acidic amino acid residues, more preferably yet at least about 80% acidic amino acid residues, more preferably yet at least about 90% acidic amino acid residues, more preferably yet at least about 95% acidic amino acid residues, and most preferably at least about 98% acidic amino acid residues. Acidic amino acid residues include aspartic acid and glutamic acid. An example of such an LBP polypeptide is BHF-1, which is a 20 amino acid length fragment of human or rabbit LBP-1 which contains amino acid residues 14 through 33. See Fig. 9 (SEQ ID NO:9). 45% of the amino acid residues of BHF-1 are acidic. The invention also includes biologically active fragments and analogs of BHF-1.

Other preferred acidic regions from the LBPs are amino acid residues 8 through 22 (SEQ ID NO:19), 8 through 33 (SEQ ID NO:20), 23 through 33 (SEQ ID NO:21), and 208 through 217 (SEQ ID NO:22) of human LBP-2 as depicted in Fig. 7 (SEQ. ID NO:7); amino acid residues 14 through 43 (SEQ ID NO:23) and 38 through 43 (SEQ ID NO:24) of rabbit or human LBP-1 as depicted in Fig. 1 (SEQ ID NO:1) and Fig. 6 (SEQ ID NO:6); amino acid residues 105 through 120 (SEQ ID NO:25), 105 through 132 (SEQ ID NO:26), 121 through 132 (SEQ ID NO:27), and 211 through 220 (SEQ ID NO:28) of rabbit LBP-2 as depicted in Fig. 2 (SEQ ID NO:2); amino acid residues 96 through 110 (SEQ ID NO:29) of rabbit LBP-3 as depicted in Fig. 5 (SEQ ID NO:5); and amino acid residues 53-59 (SEQ ID NO:41) of human LBP-3 as depicted in Fig. 8 (SEQ ID NO:8). The invention is also meant to include biologically active fragments and analogs of any of these polypeptides.

Other examples of agents include homopolymers and heteropolymers of any amino acid or amino acid analog. In certain preferred embodiments, the agent is a homopolymer of an acidic amino acid or analog thereof. In certain embodiments, the agent is a heteropolymer of one or more acidic amino acids and one or more other amino acids, or analogs thereof. For example, agents include poly(glu), poly(asp), poly(glu asp), poly(glu N), poly(asp N) and poly(glu asp N). By N is meant any amino acid, or analog thereof, other than glu or asp. By poly(glu asp) is meant all permutations of glu and asp for a given length peptide. A preferred peptide is poly(glu) of no more than about 10 amino acids in length, preferably about 7 amino acids in length.

In certain preferred embodiments, the agent is an LBP nucleic acid or a biologically active fragment or analog thereof, e.g., a nucleic acid encoding LBP-1, LBP-2 or LBP-3 polypeptide, or a biologically active fragment or analog thereof. The agent can be, e.g., a nucleic acid comprising a nucleotide sequence as set forth in SEQ ID NOS:10-18. In other embodiments, the agent is an antisense molecule, e.g., one which can bind to an LBP gene sequence.

Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the atherosclerosis. Administration of the agent can be accomplished by any method which allows the agent to reach the target cells. These methods include, e.g., injection, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the agent is obtained. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g., microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the agent with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

Administration of the agent can be alone or in combination with other therapeutic agents. In certain embodiments, the agent can be combined with a suitable carrier, incorporated into a liposome, or incorporated into a polymer release system.

In certain embodiments of the invention, the administration can be designed so as to result in sequential exposures to the agent over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the agent by one of the methods described above, or alternatively, by a controlled release delivery system in which the agent is delivered to the animal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the agent does not occur immediately upon administration, but rather is delayed for some time. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g., by long acting oral dosage forms, bolus injections, transdermal patches or subcutaneous implants.

Examples of systems in which release occurs in bursts include, e.g., systems in which the

agent is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimulus, e.g., temperature, pH, light, magnetic field, or a degrading enzyme, and systems in which the agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the agent is gradual and continuous include, e.g., erosional systems in which the agent is contained in a form within a matrix, and diffusional systems in which the agent permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

The agent can be suspended in a liquid, e.g., in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases water or an organic liquid can be used.

The agent can be administered prior to or subsequent to the appearance of atherosclerosis symptoms. In certain embodiments, the agent is administered to patients with familial histories of atherosclerosis, or who have phenotypes that may indicate a predisposition to atherosclerosis, or who have been diagnosed as having a genotype which predisposes the patient to atherosclerosis, or who have other risk factors, e.g., hypercholesterolemia, hypertension or smoking.

The agent is administered to the animal in a therapeutically effective amount. By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing atherosclerosis. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the species of animal, the animal's size, the animal's age, the agent used, the type of delivery system used, the time of administration relative to the onset of atherosclerosis symptoms, and whether a single, multiple, or controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Preferably, the concentration of the agent is at a dose of about 0.1 to about 1000 mg/kg body weight/day, more preferably at about 0.1 to about 500 mg/kg/day, more preferably yet at about 0.1 to about 100 mg/kg/day, and most preferably at about 0.1 to about 5 mg/kg/day. The specific concentration partially depends upon the particular agent used, as some are more effective than others. The dosage concentration of the agent that is actually administered is dependent at least in part upon the final concentration that is desired at the site of action, the

method of administration, the efficacy of the particular agent, the longevity of the particular agent, and the timing of administration relative to the onset of the atherosclerosis symptoms. Preferably, the dosage form is such that it does not substantially deleteriously affect the animal. The dosage can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In certain embodiments, various gene constructs can be used as part of a gene therapy protocol to deliver nucleic acids encoding an agent, e.g., either an agonistic or antagonistic form of an LBP polypeptide. For example, expression vectors can be used for in vivo transfection and expression of an LBP polypeptide in particular cell types so as to reconstitute the function of, or alternatively, abrogate the function of, LBP polypeptide in a cell in which non-wild type LBP is expressed. Expression constructs of the LBP polypeptide, and mutants thereof, may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the LBP gene to cells in vivo. Approaches include, e.g., insertion of the subject gene in viral vectors including, e.g., recombinant retroviruses, adenovirus, adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors infect or transduce cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin™ (Life Technologies, Inc., Gaithersburg, MD) or derivatized (e.g. antibody conjugated), polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $Ca_3(PO_4)_2$ precipitation carried out in vivo. The above-described methods are known to those skilled in the art and can be performed without undue experimentation. Since transduction of appropriate target cells represents the critical first step in gene therapy, choice of the particular gene delivery system will depend on such factors as the phenotype of the intended target and the route of administration, e.g., locally or systemically. Administration can be directed to one or more cell types, and to one or more cells within a cell type, so as to be therapeutically effective, by methods that are known to those skilled in the art. In a preferred embodiment, the agent is administered to arterial wall cells of the animal. For example, a genetically engineered LBP gene is administered to arterial wall cells. In certain embodiments, administration is done in a prenatal animal or embryonic cell. It will be recognized that the particular gene construct provided for in in vivo transduction of LBP expression is also useful for in vitro transduction of cells, such as for use in the diagnostic assays described herein.

In certain embodiments, therapy of atherosclerosis is performed with antisense nucleotide analogs of the genes which code for the LBPs. Preferably, the antisense nucleotides have non-hydrolyzable "backbones," e.g., phosphorothioates, phosphorodithioates or methylphosphonates. The nucleoside base sequence is complementary to the sequence of a portion of the gene coding for, e.g., LBP-1, 2 or 3. Such a sequence might be, e.g., ATTGGC if the gene sequence for the LBP is TAACCG. One embodiment of such therapy would be incorporation of an antisense analog of a portion of one of the LBP genes in a slow-release medium, e.g., polyvinyl alcohol, which is administered, e.g., by subcutaneous injection, so as to release the antisense nucleotide analog over a period of weeks or months. In another embodiment, the antisense analog is incorporated into a polymeric matrix, e.g., polyvinyl alcohol, such that the gel can be applied locally to an injured arterial wall to inhibit LBP synthesis and prevent LDL accumulation, e.g., after angioplasty or atherectomy.

The invention also includes a method for treating an animal at risk for atherosclerosis. An animal at risk for atherosclerosis is provided. An agent capable of altering an aspect of LBP structure or metabolism is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the animal occurs. Being at risk for atherosclerosis can result from, e.g., a family history of atherosclerosis, a genotype which predisposes to atherosclerosis, or phenotypic symptoms which predispose to atherosclerosis, e.g., having hypercholesterolemia, hypertension or smoking.

The invention also includes a method for treating a cell having an abnormality in structure or metabolism of LBP. A cell having an abnormality in structure or metabolism of LBP is provided. An agent capable of altering an aspect of LBP structure or metabolism is provided. The agent is administered to the cell in a therapeutically effective amount such that treatment of the cell occurs.

In certain embodiments, the cell is obtained from a cell culture or tissue culture or an embryo fibroblast. The cell can be, e.g., part of an animal, e.g., a natural animal or a non-human transgenic animal. Preferably, the LBP is LBP-1, LBP-2 or LBP-3.

The invention also includes a pharmaceutical composition for treating atherosclerosis in an animal comprising a therapeutically effective amount of an agent, the agent being capable of altering an aspect of LBP metabolism or structure in the animal so as to result in treatment of the atherosclerosis, and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, e.g., saline, liposomes and lipid emulsions.

In certain preferred embodiments, the agent of the pharmaceutical composition is an LBP polypeptide, e.g., LBP-1, LBP-2 or LBP-3, or a biologically active fragment or analog thereof. The agent can be, e.g., the polypeptide as set forth in SEQ ID NOS:1-9. Preferably, the agent is a polypeptide of no more than about 100 amino acid residues in length, more preferably of no more than about 50 amino acid residues, more preferably yet of no more than about 30 amino acid residues, more preferably yet of no more than about 20 amino acid residues, more preferably yet of no more than about 10 amino acid residues, more preferably yet of no more than about 5 amino acid residues, more preferably yet of no more than about 4 amino acid residues, more preferably yet of no more than about 3 amino acid residues, and most preferably of no more than about 2 amino acid residues. Preferably, the polypeptide includes at least about 20% acidic amino acid residues, more preferably yet at least about 40% acidic amino acid residues, more preferably yet at least about 60% acidic amino acid residues, more preferably yet at least about 80% acidic amino acid residues, more preferably yet at least about 90% acidic amino acid residues, more preferably yet at least about 95% acidic amino acid residues, and most preferably at least about 98% acidic amino acid residues.

In certain preferred embodiments, the agent is an LBP nucleic acid, e.g., a nucleic acid encoding LBP-1, LBP-2 or LBP-3 polypeptide, or a biologically active fragment or analog thereof. The agent can be, e.g., a nucleic acid comprising a nucleotide sequence as set forth in SEQ ID NOS:10-18.

The invention also includes a vaccine composition for treating atherosclerosis in an animal comprising a therapeutically effective amount of an agent, the agent being capable of altering an aspect of LBP metabolism or structure in the animal so as to result in treatment of the atherosclerosis, and a pharmaceutically acceptable carrier.

The invention also includes a method for diagnosing atherosclerotic lesions in an animal. An animal is provided. A labeled agent capable of binding to LBP present in atherosclerotic lesions is provided. The labeled agent is administered to the animal under conditions which allow the labeled agent to interact with the LBP so as to result in labeled LBP. The localization or quantification of the labeled LBP is determined by imaging so as to diagnose the presence of atherosclerotic lesions in the animal.

Preferably, the LBP is LBP-1, LBP-2 or LBP-3. The imaging can be performed by standard methods known to those skilled in the art, including, e.g., magnetic resonance imaging, gamma camera imaging, single photon emission computed tomographic (SPECT) imaging, or

positron emission tomography (PET).

Preferably, agents that bind tightly to LBPs in atherosclerotic lesions are used for atherosclerotic imaging and diagnosis. The agent is radiolabeled with, e.g., ^{99m}Tc or another isotope suitable for clinical imaging by gamma camera, SPECT, PET scanning or other similar technology. Since LBPs occur in very early lesions, such imaging is more sensitive than angiography or ultrasound for locating very early lesions which do not yet impinge on the arterial lumen to cause a visible bulge or disturbed flow. In addition to locating both early and more developed lesions, the imaging agents which bind to LBPs can also be used to follow the progress of atherosclerosis, as a means of evaluating the effectiveness of both dietary and pharmacological treatments.

Thus, a diagnostic embodiment of the invention is the adaptation of, e.g., a peptide complementary to one of the LBPs, by radiolabeling it and using it as an injectable imaging agent for detection of occult atherosclerosis. The peptide is selected from those known to bind to LBPs, e.g., RRRRRRR or KKLKLXX, or any other polycationic peptide which binds to the highly electronegative domains of the LBPs. For extracorporeal detection with a gamma scintillation (Anger) camera, technetium-binding ligands, e.g., CGC, GGCGC, or GGCGCF, can be incorporated into the peptides at the N-terminus or C-terminus for ^{99m}Tc labeling. For external imaging by magnetic resonance imaging (MRI), e.g., the gadolinium-binding chelator, diethylene triamine penta-acetic acid (DTPA), is covalently bound to the N- or C-terminus of the peptides. In yet other embodiments, the LBP-binding peptides are covalently bound, e.g., to magnetic ion oxide particles by standard methods known to those skilled in the art, e.g., conjugating the peptides with activated polystyrene resin beads containing magnetic ion oxide.

The invention also includes a method for immunizing an animal against an LBP, e.g., LBP-1, LBP-2 or LBP-3, or fragment or analog thereof. An animal having LDL is provided. An LBP or fragment or analog thereof is provided. The LBP or fragment or analog thereof is administered to the animal so as to stimiulate antibody production by the animal to the LBP or fragment or analog thereof such that binding of the LBP to the LDL is altered, e.g., decreased or increased.

The invention also includes a method of making a fragment or analog of LBP polypeptide, the fragment or analog having the ability to bind to modified LDL and native LDL. An LBP polypeptide is provided. The sequence of the LBP polypeptide is altered. The altered LBP polypeptide is tested for the ability to bind to modified LDL, e.g., methylated LDL,

oxidized LDL, acetylated LDL, cyclohexanedione-treated LDL (CHD-LDL), and to native LDL.

The fragments or analogs can be generated and tested for their ability to bind to these modified LDLs and to native LDL, by methods known to those skilled in the art, e.g., as described herein. Preferably, they are tested for their ability to bind to methylated LDL and native LDL. The binding activity of the fragment or analog can be greater or less than the binding activity of the native LBP. Preferably, it is greater. In preferred embodiments, the LBP is LBP-1, LBP-2 or LBP-3.

The invention also includes a method for isolating a cDNA encoding an LBP. A cDNA library is provided. The cDNA library is screened for a cDNA encoding a polypeptide which binds to native LDL and modified LDL, e.g., methylated LDL or oxidized LDL. The cDNA which encodes this polypeptide is isolated, the cDNA encoding an LBP.

The following non-limiting examples further illustrate the present invention.

EXAMPLES

Example 1: Construction of a Rabbit cDNA Library

This example illustrates the construction of a rabbit cDNA library using mRNA from balloon-deendothelialized healing rabbit abdominal aorta. Balloon-catheter deendothelialized rabbit aorta has been shown to be a valid model for atherosclerosis (Minick et al., Am. J. Pathol. 95:131-158 (1979).

The mRNA was obtained four weeks after ballooning to maximize focal LDL binding in the ballooned rabbit aorta. First strand cDNA synthesis was carried out in a 50 µl reaction mixture containing 4 µg mRNA; 2 µg oligo d(T) primer; methylation dNTP mix (10 mM each); 10 mM DTT; 800 units superscript II RT (Life Technologies, Gaithersburg, MD); 1 X first strand cDNA synthesis buffer (50 mM Tris-HCl, pH 8.3; 75 mM KCl; 5 mM $MgCl_2$), which was incubated for 1 hr at 37°C. The reaction mixture was then adjusted to 250 µl through the addition of 1 X second strand buffer (30 mM Tris-HCl, pH 7.5; 105 mM KCl; 5.2 mM $MgCl_2$); 0.1 mM DTT; methylation dNTP mix (10 mM each); 50 units E. coli DNA polymerase I, 3 units RNase H; 15 units E. coli DNA ligase (all enzymes from Life Technologies), which was incubated for an additional 2.5 hr at 15°C. The resulting double-stranded cDNAs (dscDNA) were then treated with 1.5 units T4 DNA polymerase (Novagen Inc., Madison, WI) for 20 min at 11°C to make blunt-ended dscDNA. These were then concentrated by ethanol precipitation and EcoR1/Hind III linkers were attached to the ends by T4 DNA ligase (Novagen Inc.). The linker-ligated cDNAs were treated with EcoR1 and HindIII restriction enzymes to produce EcoR1 and

Hind III recognition sequences at their 5' and 3' ends, respectively. After the removal of linker DNA by gel exclusion chromatography, the dscDNAs were inserted into λEXlox phage arms (Novagen Inc.) in a unidirectional manner by T4 DNA ligase and packaged into phage particles according to the manufacturer's protocol (Novagen Inc.). A phage library of cDNAs containing 2 x 10^6 independent clones was established from 4 μg of mRNA.

Example 2: Identification of Rabbit cDNAs Encoding LDL Binding Proteins (LBPs)

This example illustrates a method of functionally screening a rabbit cDNA library so as to identify cDNAs encoding LBPs which bind to both native LDL and methyl LDL. Methyl LDL is not recognized by previously reported cell surface receptors. See, e.g., Weisgraber et al., J. Biol. Chem. 253:9053-9062 (1978).

A fresh overnight culture of E. coli ER1647 cells (Novagen Inc.) was infected with the cDNA phage obtained from Example 1, and plated at a density of 2 x 10^4 plaque-forming units (pfu) in 150 mm diameter plates containing 2 X YT agar. A total of 50 plates, equivalent to 1 x 10^6 phage, were plated and incubated at 37°C until the plaques reached 1 mm in diameter (5-6 hr). A dry nitrocellulose membrane, which had previously been saturated with 10 mM IPTG solution, was layered on top of each plate to induce the production of recombinant protein, as well as to immobilize the proteins on the membranes. The plates were incubated at 37°C for an additional 3-4 hr, and then overnight at 4°C.

The next day, the membranes were lifted from each plate and processed as follows. Several brief rinses in TBST solution (10mM Tris-HCl, pH 8.0; 150mM NaCl, 0.05% Tween 20); two 10-min rinses with 6M guanidine-HCl in HBB (20mM HEPES, pH 7.5; 5mM $MgCl_2$, 1mM DTT, and 5mM KCl); two 5-min rinses in 3M guanidine-HCl in HBB; a final brief rinse in TBSEN (TBS, 1mM EDTA, 0.02% NaN_3).

The membranes were then blocked for 30 min at room temperature in a solution of TBSEN with 5% non-fat dry milk, followed by 10 min in TBSEN with 1% non-fat dry milk. Following blocking, the membranes were incubated with native human LDL (obtained as described in Example 11 or methylated human LDL (meLDL) (see Weisgraber et al., J. Biol. Chem. 253:9053-9062 (1978)), at a concentration of 4 μg/ml, in a solution containing 1 X TBSEN, 1% non-fat dry milk, 1mM PMSF, 0.5 X protease inhibitor solution (1mM ε-amino caproic acid/1mM benzamidine). Incubation was for 4 hr at room temperature in a glass Petri dish with gentle stirring on a stirring table, followed by overnight at 4°C with no stirring.

Specifically bound meLDL and native LDL were detected on the nitrocellulose membranes by antibodies against human LDL. Sheep anti-human LDL polyclonal antibodies (Boehringer Mannheim, Indianapolis, IN) were adsorbed with E. coli plys E cell extracts to abolish background. For adsorption, E. coli plys E cells were grown to log phase, spun down and resuspended in PBS containing 1 mM PMSF, 2 mM ϵ-amino caproic acid, and 1 mM benzamidine. The cell suspension then underwent 8 freeze-thaw cycles via immersion in liquid nitrogen and cold running tap water, respectively. The anti LDL antibodies/cell extract solution were incubated with gentle stirring for 1 hr at 4°C (1 ml of antibody solution/3 mg crude cell extract). Following incubation, the mixture was centrifuged (10,000 x g; 10 min; 4°C) and the supernatant was stored at 4°C in the presence of 0.02% NaN_3 until use. The membranes were processed for immunoscreening as follows: (i) three 5-min washes at room temperature in TBSEN containing 1% gelatin; (ii) 30 min incubation in PBS, pH 7.4 with 1% gelatin; (iii) two-hr room temperature incubation with gentle stirring in fresh PBS gelatin solution containing adsorbed sheep anti-human LDL antibodies (Boehringer Manheim, Indianapolis, IN) (1:1000 dilution); (iv) three brief washes in TBS, pH 7.4; (v) one-hr room temperature incubation with gentle stirring in PBS/gelatin solution containing donkey antisheep alkaline phosphatase-conjugated antibodies (Sigma, St. Louis, MO) (1:10,000 dilution); (vi) three brief washes with TBS, pH 7.4.; and (vii) development according to the manufacturer's instructions, using an alkaline phosphatase substrate development kit (Novagen Inc.). Phage plaques which produced LBPs appeared as blue-colored "donuts" on the membranes.

The phage from Example 1 containing the LBP cDNAs were plaque-purified and converted into plasmid subclones by following a protocol called "Autosubcloning by Cre-mediated Plasmid Excision" provided by Novagen Inc. DNA sequences were obtained by the dideoxynucleotide chain-termination method (Sanger et al., Proc. Natl. Acad. Sci., USA 74:5463-5467 (1977), and analyzed by an Applied Biosystems automated sequencer. The open reading frame (ORF) of each cDNA was determined from consensus sequences obtained from both the sense and antisense strands of the cDNAs. Sequencing confirmed that three previously unknown genes had been isolated. Since the genes were selected by functional screening for LDL binding, the proteins coded by these genes were termed LDL binding proteins (LBPs), specifically, LBP-1, LBP-2 and LBP-3. The cDNA sequences for rabbit LBP-1, LBP-2 and LBP-3 and the corresponding proteins are set forth in SEQ ID NOS:10-14.

Based on their respective cDNA coding sequences, the sizes of the recombinant proteins were determined to be 16.2 kDa for LBP-1, 40 kDa for LBP-2, and 62.7 kDa for LBP-3.

Example 3: Northern Blot Analysis of Rabbit RNA Using LBP cDNA or cRNA

This example illustrates the size and tissue distribution of LBP mRNAs. Total RNA was isolated from different rabbit tissues: adrenals, thoracic aorta, abdominal aorta, ballooned and reendothelialized abdominal aorta, heart, kidney, smooth muscle cells, lung and liver, by Trizol reagent (Life Technologies) and concentrated by ethanol precipitation. Gel electrophoresis of RNA was carried out in 1.2% agarose gel containing 1 X MOPS buffer (0.2M MOPS, pH 7.0; 50mM sodium acetate; 5mM EDTA, pH 8.0) and 0.37M formaldehyde. Gels were loaded with 20 μg total RNA from each tissue examined and electrophoresed at 100 volts for 2 hr in 1 X MOPS buffer. RNAs were blotted onto supported nitrocellulose membranes (Schleicher & Schuell, Keene, NH) and immobilized by baking at 80°C for 2 hr. Hybridization to radiolabeled LBP-1, LBP-2 and LBP-3 cDNA or cRNA probes was carried out by standard procedures known to those skilled in the art (see, e.g., Ausubel et al., Current Protocols in Molecular Biology; John Wiley & Sons (1989)); signals were detected by autoradiography.

The results were as follows: the sizes of the mRNAs were about 1.3 kb for LBP-1, about 2.3-2.5 kb for LBP-2, and about 4.7 kb for LBP-3. LBP-1, LBP-2 and LBP-3 mRNA were found in all tissues tested, but the highest amount was in ballooned abdominal aorta.

Example 4: Isolation of Human LBP cDNAs

This example illustrates isolation of human LBP cDNAs. Human LBP cDNA clones were isolated from three cDNA libraries. A human fetal brain cDNA library was obtained from Stratagene, LaJolla, CA, a human liver and a human aorta cDNA library were obtained from Clontech, Palo Alto, CA, and screened with a radiolabeled cDNA probe derived from rabbit LBP-1, LBP-2 or LBP-3, according to the method described in Law et al., Gene Expression 4:77-84 (1994). Several strongly hybridizing clones were identified and plaque-purified. Clones were confirmed to be human LBP-1, LBP-2 and LBP-3, by DNA sequencing using the dideoxynucleotide chain-termination method and analysis by an Applied Biosystems automated sequencer. The cDNA sequences and the corresponding proteins for human LBP-1, LBP-2 and LBP-3 are set forth in SEQ ID NOS:15, 16 and 17, respectively. A comparison between the corresponding LBP-1, LBP-2 and LBP-3 protein sequences for rabbit and human are shown in Figs. 19, 20 and 21.

Example 5: Isolation of Recombinant LBP-1, LBP-2 and LBP-3 Rabbit Proteins from E. coli

LBP cDNA was isolated from the original pEXlox plasmids obtained as described in Examples 1 and 2, and subcloned into the pPROEX-HT vector (Life Technologies) for recombinant protein expression. Induction of the recombinant protein by IPTG addition to transformed E. coli DH10B cultures resulted in the expression of recombinant protein containing a 6-histidine tag (N-terminal). This tagged protein was then purified from whole cell proteins by binding to Ni-NTA (nickel nitrilo-triacetic acid) as described in the protocol provided by the manufacturer (Qiagen, Inc., Santa Clara, CA). The preparation obtained after the chromatography step was approximately 90% pure; preparative SDS-PAGE was performed as the final purification step.

When required by the characterization procedure, iodination of LBPs was carried out using Iodobeads (Pierce, Rockford, IL). The Iodobeads were incubated with 500 μCi of $Na^{125}I$ solution (17 Ci/mg) (New England Nuclear, Boston, MA) in a capped microfuge tube for 5 min at room temperature. The protein solution was added to the Iodobeads-$Na^{125}I$ microfuge tube and incubated for 15 min at room temperature. At the end of this incubation, aliquots were removed for the determination of total soluble and TCA precipitable counts. The radiolabeled protein was then precipitated with cold acetone (2.5 vol; -20°C; 2.5 hr). Following this incubation, precipitated protein was collected by centrifugation (14,000 g; 1 hr; room temperature) and resuspended in sample buffer (6 M urea/50 mM Tris, pH 8.0/2 mM EDTA). Integrity of the protein preparation was assessed by SDS-PAGE.

The identities of the recombinant LBPs were confirmed using standard protein sequencing protocols known to those skilled in the art. (A Practical Guide for Protein and Peptide Purification for Microsequencing, Matsudaira, ed., Academic Press, Inc., 2d edition (1993)). Analysis was performed using an Applied Biosystems Model 477A Protein Sequencer with on-line Model 120 PTH amino acid analyzer.

Example 6: Production of Antibodies to LBP-1, LBP-2 and LBP-3

This example illustrates the production of polyclonal antibodies to LBP-1, LBP-2 and LBP-3. A mixture of purified recombinant LBP protein (0.5 ml; 200 μg) and RIBI adjuvant (RIBI ImmunoChem Research, Inc., Hamilton, MT) was injected subcutaneously into male guinea pigs (Dunkin Hartley; Hazelton Research Products, Inc., Denver, PA) at 3-5 sites along the dorsal thoracic and abdominal regions of the guinea pig. Blood was collected by

venipuncture on days 1 (pre-immune bleeding), 28, 49 and 70. Booster injections were administered on days 21 (100 μg; SC), 42 (50 μg; SC), and 63 (25 μg; SC). The titer of the guinea pig antiserum was evaluated by serial dilution "dot blotting." Preimmune antiserum was evaluated at the same time. After the third booster of LBP protein, the titer against the recombinant protein reached a maximal level with a detectable colorimetric response on a dot blot assay of 156 pg.

Specificity of the polyclonal antibody for recombinant LBP-1, LBP-2 or LBP-3 was demonstrated using Western blot analysis. (Towbin et al., Proc. Natl. Acad. Sci. USA 76:4350 (1979)). The protein-antibody complex was visualized immunochemically with alkaline phosphatase-conjugated goat anti-guinea pig IgG, followed by staining with nitro blue tetrazolium (BioRad Laboratories, Hercules, CA). Non-specific binding was blocked using 3% non-fat dry milk in Tris buffered saline (100 mM Tris; 0.9% NaCl, pH 7.4).

Example 7: Immunohistochemical Characterization

This example illustrates the presence of LBPs in or on endothelial cells covering plaques, in or on adjacent smooth muscle cells, and in the extracellular matrix. In addition, co-localization of LDL and LBPs was demonostrated. These results were obtained by examining ballooned rabbit arterial lesions and human atherosclerotic plaques by immunohistochemical methods.

Ballooned deendothelialized aorta was obtained from rabbits which had received a bolus injection of human LDL (3 mg; i.v.) 24 hr prior to tissue collection. Human aortas containing atherosclerotic plaques were obtained from routine autopsy specimens. Tissues were fixed in 10% buffered formalin (≤24 hr) and imbedded in paraffin using an automated tissue-imbedding machine. Tissue sections were cut (5-7 μ) and mounted onto glass slides by incubating for 1 hr at 60°C. Sections were deparaffinized. After a final wash with deionized H_2O, endogenous peroxidase activity was eliminated by incubating the sections with 1% H_2O_2/H_2O buffer for 5 min at room temperature. Sections were rinsed with phosphate buffered saline (PBS) for 5 min at room temperature and nonspecific binding was blocked with 5% normal goat serum or 5% normal rabbit serum depending on the source of the secondary antibody (Sigma, St. Louis, MO) (1 hr; room temperature). Sections were then incubated with a 1:50 dilution (in 5% normal goat serum/PBS) of a guinea pig polyclonal antibody against the rabbit form of recombinant LBP-1, LBP-2 or LBP-3. Controls included preimmune serum as well as specific antisera to LBP-1,

LBP-2, or LBP-3 in which the primary antibody was completely adsorbed and removed by incubation with recombinant LBP-1, LBP-2 or LBP-3 followed by centrifugation prior to incubation with the tissue sections. An affinity purified rabbit polyclonal antibody against human apolipoprotein B (Polysciences Inc.; Warrington, PA) was used at a dilution of 1:100 (in 5% normal rabbit serum/PBS). Sections were incubated for 2 hr at room temperature in a humidified chamber. At the end of incubation, sections were rinsed with PBS and incubated with a 1:200 dilution (in 5% normal goat serum/PBS) of goat anti-guinea pig biotinylated IgG conjugate (Vector Laboratories, Burlingame, CA) or a 1:250 dilution (in 5% normal rabbit serum/PBS) of rabbit anti-goat biotinylated IgG conjugate (Vector Laboratories, Burlingame, CA) for 1 hr at room temperature in a humidified chamber. Sections were then rinsed with PBS and antigen-antibody signal amplified using avidin/biotin HRP conjugate (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were developed using DAB substrate (4-6 min; room temperature) and counterstained with hematoxylin.

In the ballooned rabbit artery, immunohistochemistry with the anti-LBP-1, LBP-2 and LBP-3 antibodies showed that LBP-1, LBP-2 and LBP-3 were located in or on functionally modified endothelial cells at the edges of regenerating endothelial islands, the same location in which irreversible LDL binding has been demonstrated (Chang et al., Arteriosclerosis and Thrombosis 12:1088-1098 (1992)). LBP-1, LBP-2 and LBP-3 were also found in or on intimal smooth muscle cells underneath the functionally modified endothelial cells, and to a lesser extent, in extracellular matrix. No LBP-1, LBP-2 or LBP-3 was detected in still deendothelialized areas, where LDL binding had been shown to be reversible (Chang et al., Arteriosclerosis and Thrombosis 12:1088-1098 (1992)). Immunohistochemistry of ballooned rabbit aorta with anti-human apolipoprotein B antibodies showed the presence of LDL at the same locations as that found for LBP-1, LBP-2 and LBP-3.

In the human atherosclerotic plaques taken at routine autopsies, immunohistochemistry with the anti-LBP-1, anti-LBP-2 and anti-LBP-3 antibodies showed that LBP-1, LBP-2, and LBP-3 were also found in or on endothelial cells covering plaques and in or on adjacent smooth muscle cells. In the human tissue, there was greater evidence of LBP-1, LBP-2 and LBP-3 in extracellular matrix.

The results obtained with paraffin sections were identical to those of frozen sections.

Example 8: Affinity Coelectrophoresis (ACE) Assays of LBPs and LDL or HDL

This example illustrates that binding occurs between LBP-1, LBP-2 or LBP-3 and LDL, and that this binding is specific, as illustrated by the fact that binding does not occur between LBP-1, LBP-2 or LBP-3 and HDL (high density lipoprotein).

Analysis of the affinity and specificity of recombinant rabbit LBP-1, LBP-2 or LBP-3 binding to LDL was carried out using the principle of affinity electrophoresis (Lee and Lander, Proc. Natl. Acad. Sci. USA 88:2768-2772 (1991)). Melted agarose (1%; 65°C) was prepared in 50 mM sodium MOPS, pH 7.0; 125 mM sodium acetate, 0.5% CHAPS. A teflon comb consisting of nine parallel bars (45 x 4 x 4 mm/3 mm spacing between bars) was placed onto GelBond film (FMC Bioproducts, Rockland, ME) fitted to a plexiglass casting tray with the long axis of the bars parallel to the long axis of the casting tray. A teflon strip (66 x 1 x 1 mm) was placed on edge with the long axis parallel to the short axis of the casting tray, at a distance of 4 mm from the edge of the teflon comb. Melted agarose (>65°C) was then poured to achieve a height of approximately 4 mm. Removal of the comb and strip resulted in a gel containing nine 45 x 4 x 4 mm rectangular wells adjacent to a 66 x 1 mm slot. LDL or HDL samples were prepared in gel buffer (50mM sodium MOPS, pH 7.0, 125 mM sodium acetate) at twice the desired concentration. Samples were then mixed with an equal volume of melted agarose (in 50 mM MOPS, pH 7.0; 125 mM sodium acetate; 50°C), pipetted into the appropriate rectangular wells and allowed to gel. The binding affinity and specificity of LBP-1 and LBP-3 was tested using several concentrations of LDL (540 to 14 nM) and HDL (2840-177 nM). A constant amount (0.003 nM - 0.016 nM) of ^{125}I-labeled LBP-1, LBP-2 or LBP-3 (suspended in 50 mM sodium MOPS, pH 7.0; 125 mM sodium acetate; 0.5% bromphenol blue; 6% (wt/vol) sucrose) was loaded into the slot. Gels were electrophoresed at 70v/2hr/20°C. At the end of the run, the gels were air dried and retardation profiles were visualized by exposure of X-ray films to the gels overnight at -70°C, with intensifying screens).

LDL retarded LBP-1, LBP-2 and LBP-3 migration through the gel in a concentration-dependent, saturable manner, indicating that LBP-1, LBP-2 and LBP-3 binding to LDL was highly specific. This conclusion is supported by the fact that HDL did not retard LBP-1, LBP-2 or LBP-3. A binding curve generated from the affinity coelectrophoresis assay indicated that LBP-1 binds to LDL with a K_d of 25.6 nM, that LBP-2 (rabbit clone 26) binds to LDL with a K_d of 100 nM, and that LBP-3 (80 kDa fragment) binds to LDL with a K_d of 333 nM.

In addition to testing affinity and specificity of LBP-1, LBP-2 and LBP-3 binding to

LDL, the ability of "cold" (i.e., non-radiolabeled) LBP-1, LBP-2 or LBP-3 to competitively inhibit radiolabeled LBP-1, LBP-2 or LBP-3 binding to LDL, respectively, was tested. Competition studies were carried out using fixed concentrations of cold LDL and radiolabeled LBP-1 and increasing amounts of cold recombinant LBP-1 (6-31 μM). The ACE assay samples and gel were prepared as described herein. Cold LBP-1 inhibited binding of radiolabeled LBP-1 to LDL in a concentration-dependent manner, cold LBP-2 inhibited binding of radiolabeled LBP-2 to LDL in a concentration-dependent manner, and cold LBP-3 inhibited binding of radiolabeled LBP-3 to LDL in a concentration-dependent manner.

Rabbit and human LBP-2 contain a long stretch of acidic amino acids at the amino terminal (rabbit LBP-2 amino acid residues 105 through 132 and human LBP-2 amino acid residues 8 through 33). The possibility that this segment of LBP-2 was the LDL binding domain was tested by subcloning two rabbit LBP-2 clones which differ from each other by the presence or absence of this acidic region (clone 26 and clone 45, respectively) into expression vectors, by standard methods known to those skilled in the art. ACE assays were then conducted in order to assess the affinity and specificity of the binding of these two clones to LDL. LDL retarded clone 26 derived radiolabeled LBP-2 migration through the gel in a concentration-dependent, saturable, manner while clone 45 derived radiolabeled LBP-2 migration was not retarded.

Competition studies using fixed concentrations of cold LDL and clone 26 derived radiolabeled LBP-2 and increasing concentrations of cold recombinant LBP-2/clone 26 and LBP-2/clone 45 were carried out. Cold clone 26 derived LBP-2 inhibited binding of clone 26 derived radiolabeled LBP-2 to LDL in a concentration-dependent manner. Clone 45 derived LBP-2, on the other hand, did not affect the binding of clone 26 derived radiolabeled LBP-2 to LDL. These results indicate that the long stretch of acidic amino acids contain a binding domain of LBP-2 to LDL.

Example 9: Affinity Coelectrophoresis (ACE) Assays of LBP-1 or LBP-2 and LDL in the Presence of Inhibitors

This example illustrates that binding between LBP-1 or LBP-2 and LDL is inhibited by polyglutamic acid or BHF-1. The ability of a third compound to inhibit binding between two proteins previously shown to interact was tested by a modification of the ACE assays described in Example 8. The third compound was added to the top or wells together with the radiolabeled protein. If the third compound inhibited binding, the radiolabeled protein would run through the

gel. If the third compound did not inhibit binding, migration of the radio-labeled protein was retarded by the protein cast into the gel.

Inhibition of LBP-1/LDL or LBP-2/LDL binding by polyglutamic acid (average MW about 7500, corresponding to about 7 monomers) was shown by casting a constant amount of LDL (148 nM) in all the rectangular lanes. A constant amount (1 µl) of ^{125}I-labeled LBP-1 or LBP-2 (0.003 nM - 0.016 nM) was loaded in the wells at the top of the gel, together with increasing concentrations of polyglutamic acid (obtained from Sigma) (0-0.4 nM). The gel was electrophoresed at 70 volts for 2 hr, dried and placed on X-ray film, with intensifying screens, overnight at -70°C before the film was developed to determine the retardation profile of LBP-1 and LBP-2. As the concentration of polyglutamic acid increased, retardation of radiolabeled LBP-1 and LBP-2 migration by LDL decreased in a concentration-dependent manner, which showed that polyglutamic acid inhibited binding between LBP-1, LBP-2 and LDL.

Inhibition of LBP-1/LDL binding by BHF-1 was shown by casting a constant amount of LDL (148 nM) in all the rectangular lanes. A constant amount of ^{125}I-labeled LBP-1 (0.003 nM - 0.016 nM) was loaded in the wells at the top of the gel, together with increasing concentrations of BHF-1 (0-10 nM), obtained as described in Example 15. The gel was electrophoresed at 70 volts for 2 hr, dried and placed on X-ray film, with intensifying screens, overnight at -70°C. The film was then developed to determine the retardation profile of ^{125}I-LBP-1. As the concentration of BHF-1 increased, retardation of LBP-1 by LDL decreased in a concentration-dependent manner, which demonstrated that BHF-1 inhibited binding between LBP-1 and LDL.

Example 10: Affinity Coelectrophoresis (ACE) Assays for Identifying Fragments, Analogs and Mimetics of LBPs which Bind to LDL

This example illustrates a method for identifying fragments, analogs or mimetics of LBPs which bind to LDL, and which thus can be used as inhibitors of LDL binding to LBP in the arterial walls, by occupying binding sites on LDL molecules, thereby rendering these sites unavailable for binding to LBP in the arterial wall.

Fragments of LBPs are generated by chemical cleavage or synthesized from the known amino acid sequences. Samples of these fragments are individually added (cold) to radiolabeled LBP as described in Example 8, to assess the inhibitory potency of the various fragments. By iterative application of this procedure on progressively smaller portions of fragments identified as inhibitory, the smallest active polypeptide fragment or fragments are identified. In a similar

manner, analogs of the LBPs are tested to identify analogs which can act as inhibitors by binding to LDL. And, similarly, mimetics of LBP (molecules which resemble the conformation and/or charge distributions of the LDL-binding sites on LBP molecules) are tested in a similar fashion to identify molecules exhibiting affinities for the LDL-binding sites on LBP.

The affinities of the inhibitors so identified are at least as strong as the affinity of LDL itself for the LDL-binding sites on LBP. The inhibitors bind at least competitively, and some irreversibly and preferentially as well, to the LDL-binding sites, thereby rendering such sites unavailable for binding to humoral LDL.

Example 11: ELISA Assays

This example illustrates the use of an ELISA plate assay for the quantification of a test compound's capacity to inhibit the binding of LDL to a specific LBP.

The assay was carried out as follows: LDL was diluted in 50 mM Na_2HCO_3, pH 9.6/0.02% NaN_3 and added to the wells of a 96-well plate (ImmunoWare 96-Well Reacti-Bind EIA Polystyrene Plates; Pierce (Rockford, IL)) to achieve a final concentration ranging from 0.1 to 1 µg/well. The plates were incubated for 6 hr at room temperature. At the end of the incubation period, the wells were washed 3 times with Tris-buffered saline, pH 7.4 (TBS), and blocked overnight with 200 µl of 1% bovine serum albumin (BSA) in TBS/0.02% NaN_3 (Sigma; St. Louis MO) at room temperature. The wells were then incubated with 200 µl of LBP protein (5-10 µg/well) in TBS and varying concentrations of the test compound. Plates were incubated for 1 hr at room temperature. The wells were then washed three times with TBS and blocked for 2 hr with 200 µl of 1% BSA in TBS/0.02% NaN_3 at room temperature. At the end of the incubation period, the wells were washed 3 times with TBS and a 1:1000 dilution (in TBS/0.05% Tween 20) of the appropriate guinea pig anti-LBP protein polyclonal antibody was added to the wells and incubated for 1 hr at room temperature. The wells were then washed 3 times with TBS/0.05% Tween 20; a 1:30,000 dilution of goat anti-guinea pig IgG alkaline phophatase conjugate (Sigma) was added to each well. Plates were incubated for 1 hr at room temperature. The wells were washed 3 times with TBS/0.05% Tween 20 and a colorimetric reaction was carried out by adding 200 ml of p-nitrophenyl phosphate substrate (Sigma; St. Louis MO) to the wells. The reaction was allowed to proceed for 30 min at room temperature and stopped with 50 µl of 3N NaOH. The absorbance was determined at 405 nm using an ELISA plate reader. The test compound's effectiveness in blocking the binding of LDL to the recombinant protein was

assessed by comparing the absorbance values of control and treated groups.

Alternatively, LBPs, rather than LDL, were bound to the plate. Recombinant LBP protein binding to LDL and the effect of varying concentration of the inhibitor on LBP-LDL binding was determined through the use of antibodies against LDL. This interaction was visualized through the use of a secondary antibody conjugated to a reporter enzyme (e.g. alkaline phosphatase).

ELISA plate assays were used to screen agents which can affect the binding of LBP proteins to LDL. For example, peptides derived from LBP-1 and human LBP-3 protein sequences (BHF-1 and BHF-2, respectively) were synthesized and have been shown to reduce the binding of LDL to recombinant LBP-1 and LBP-2 in this format. These results were in agreement with those obtained with the ACE assays.

Example 12: Administration of Humanized Antibodies Against LBPs so as to Block LDL-Binding Sites on the LBPs

This example illustrates administration to patients of humanized antibodies against LBP-1, LBP-2 or LBP-3 so as to block LDL-binding sites on arterial LBP molecules. Mouse monoclonal antibodies are humanized by recombinant DNA techniques and produced by standard procedures known to those skilled in the art (Berkower, I., Curr. Opin. Biotechnol. 7:622-628 (1996); Ramharayan and Skaletsky, Am. Biotechnol. Lab 13:26-28 (1995)) against LBPs and/or the LDL-binding sites on the LBPs. The corresponding Fab fragments are also produced, as described in Goding, J.W., Monoclonal Antibodies:Principles and Practice, Academic Press, New York, NY (1986). These antibodies are administered parenterally in sufficient quantity so as to block LDL-binding sites on the LBP molecules, i.e., 1-10 mg/kg daily. This prevents the irreversible arterial uptake of LDL that is required to facilitate oxidation of the LDL.

Example 13: Preparation of LDL

This example illustrates the preparation of LDL. LDL was prepared from the plasma of normolipemic donors (Chang et al., Arterioscler. Thromb. 12:1088-1098 (1992)). 100 ml of whole blood was placed into tubes containing 100 mM disodium EDTA. Plasma was separated from red blood cells by low-speed centrifugation (2,000 g; 30 min; 4°C). Plasma density was adjusted to 1.025 gm/ml with a solution of KBr and centrifuged for 18-20 hr, 100,000 x g, 12°C.

Very low density lipoproteins (VLDL) were removed from the tops of the centrifuge tubes with a Pasteur pipet. The density of the infranate was raised to 1.050 gm/ml with KBr solution and centrifuged for 22-24 hr, 100,000 x g, 12° C. LDL was removed from the tops of the centrifuge tubes with a drawn out Pasteur pipet tip. Purity of the LDL preparation was checked by Ouchterlony double immunodiffusion against antibodies to human LDL, human HDL, human immunoglobulins, and human albumin. KBr was removed from the LDL solution by dialysis (1L, x 2, ≈ 16 hr) against 0.9% saline, pH 9.0, containing 1 mM EDTA and 10 μM butylated hydroxytoluene (BHT), the latter to prevent oxidation of LDL. Following dialysis, LDL protein was measured by the method of Lowry (Lowry et al., J. Biol. Chem. 193:265-275 (1951)), and the LDL was stored at 4°C until use. LDL preparations were kept for no more than 4-6 weeks.

Example 14: Preparation of HDL

This example illustrates the preparation of HDL. HDL was prepared from plasma of normolipemic donors. 100 ml of whole blood was placed into tubes containing 100 mM disodium EDTA and plasma was collected by centrifugation (2000 g; 30 min; 4°C). Apolipoprotein B containing lipropoteins present in plasma were then precipitated by the sequential addition of sodium heparin (5,000 units/ml) and $MnCl_2$ (1M) to achieve a final concentration of 200 units/ml and 0.46 M, respectively (Warnick and Albers, J. Lipid Res. 19:65-76 (1978)). Samples were then centrifuged (2000 g; 1 hr; 4°C). The supernatant was collected and density adjusted to 1.21 g/ml by the slow addition of solid KBr. HDL was separated by ultracentrifugation (100,000 g; >46 hr; 12°C). Purity of the HDL preparation was assessed via Ouchterlony double immunodiffusion test using antibodies against human HDL, human LDL, human immunoglobulins, and human albumin. HDL samples were dialyzed against saline pH 9.0/1mM EDTA/10μM BHT (4L; 24 hr/4°C) and total protein was determined by the Lowry protein assay (Lowry et al., J. Biol. Chem. 193:265-275 (1951)). HDL was stored at 4°C until use. HDL preparations were kept for no longer than 2 weeks.

Example 15: Synthesis of BHF-1

This example illustrates the synthesis of BHF-1, a fragment of human or rabbit LBP-1 which contains amino acid residues 14 through 33. BHF-1 was synthesized using an Applied Biosystems Model 430A peptide synthesizer with standard T-Boc NMP chemistry cycles. The sequence of BHF-1 is as follows:

val-asp-val-asp-glu-tyr-asp-glu-asn-lys-phe-val-asp-glu-glu-asp-gly-gly-asp-gly (SEQ ID NO:9)

After synthesis, the peptide was cleaved with hydrofluoric acid/anisole (10/1 v/v) for 30 min at -10°C and then incubated for 30 min at 0°C. BHF-1 was then precipitated and washed three times with cold diethyl ether. Amino acid coupling was monitored with the ninhydrin test (>99%).

The BHF-1 peptide was purified to homogeneity by high performance liquid chromatography on a reverse phase Vydac C_4 column (2.24 X 25 cm) using a linear gradient separation (2-98% B in 60 min) with a flow rate of 9 ml/min. Buffer A consisted of 0.1% trifluoroacetic acid (TFA)/Milli Q water and Buffer B consisted of 0.085% TFA/80% acetonitrile. The gradient was run at room temperature and absorbance monitored at 210 and 277 nm.

Fast atom bombardment-mass spectrometry gave a protonated molecular ion peak $(M+H)^+$ at m/z= 2290.2, in good agreement with the calculated value. On amino acid analysis, experimental values for the relative abundance of each amino acid in the peptide were in good agreement with theoretical values. The lyophilized peptide was stored at -20°C.

Example 16: In Vitro Screening for Agents Which Inhibit Binding Between LDL and LBPs

This example illustrates in vitro screening for agents which inhibit binding between LDL and LBPs.

A candidate polypeptide for being an agent is chosen, e.g., LBP-1, LBP-2, LBP-3, BHF-1 or any other polypeptide. The shortest fragment of the polypeptide that inhibits LDL binding to LBPs in vitro is determined. Peptides are synthesized by standard techniques described herein. Inhibition assays are performed using standard ELISA techniques for screening, and affinity coelectrophoresis (ACE) assays to confirm the ELISA results, as described herein. Short peptides ranging, e.g., from dimers to 20-mers are constructed across sequences of the candidate polypeptide whose chemical characteristics make them likely LDL binding sites, e.g., acidic regions. The ability of shorter and shorter lengths of the peptides to inhibit LDL binding in vitro and to mammalian cells in culture is tested. For example, the effect of the peptide on inhibiting LDL binding in mammalian cells transfected to express an LBP gene is tested. Each of the peptides so identified as an inhibitor is tested with each of LBP-1, LBP-2 and LBP-3, to

determine whether a single inhibitor works against all three LBPs.

Once the minimum active sequence is determined, the peptide backbone is modified so as to inhibit proteolysis, as discussed herein. For example, modification is accomplished by substitution of a sulfoxide for the carbonyl, by reversing the peptide bond, by substituting a methylene for the carbonyl group, or other similar standard methodology. See Spatola, A.F., "Peptide Backbone Modifications: A Structure-Activity Analysis of Peptides Containing Amide Bond Surrogates, Conformational Constraints, and Related Backbone Replacements," in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp. 267-357, B. Weinstein (ed.), Marcel Dekker, Inc., New York (1983). The ability of these analogs to inhibit LDL binding to the LBPs in vitro is tested by ELISA and ACE assays in a similar manner as for the natural peptides described above.

Example 17: In Vitro Screening With Cultured Mammalian Cells for Agents Which Inhibit Binding Between LDL and LPBs

This example illustrates cell-based in vitro screening of agents which have been shown by in vitro tests such as ACE assay and ELISA to be potential inhibitors of binding between LDL and LBPs.

Mammalian cells, such as 293 cells, which are commonly used for expression of recombinant gene constructs, are used to develop cell lines which express LBPs on the cell surface. This is done by subcloning LBP open reading frames (ORFs) into a mammalian expression plasmid vector, pDisplay (Invitrogen, Carlsbad, CA), which is designed to express the gene of interest on the cell surface. The use of mammalian cells to produce LBPs allows for their expression in a functionally active, native conformation. Therefore, stably transfected mammalian cell lines with surface expression of LBPs individually, or in combination, are particularly suitable for assaying and screening inhibitors that block LDL binding in cell culture, as well as to evaluate the cytotoxicity of these compounds.

Specifically, LBP ORFs are amplified by PCR (Perkin Elmer, Foster City, CA) from cDNA templates using Taq polymerase (Perkin Elmer) and appropriate primers. The amplified LBP ORFs are purified by agarose gel electrophoresis and extracted from gel slices with the Bio-Rad DNA Purification kit (Bio-Rad, Hercules, CA). The purified DNAs are then cut with the restriction enzymes Bgl II and Sal I (New England Biolabs, Beverly, MA) to generate cohesive ends, and purified again by agarose gel electrophoresis and DNA extraction as described above.

The LBP ORFs are then subcloned into the Bgl II/Sal I sites in the mammalian expression vector, pDisplay (Invitrogen) by ligation. Recombinant plasmids are established by transformation in E.coli strains TOP10 (Invitrogen) or DH5α (Life Technologies, Grand Island, NY). Recombinant pDisplay/LBP plasmid DNA is isolated from overnight E.coli cultures with the Bio-Rad Plasmid Miniprep kit, cut with Bgl II/Sal I, and analyzed by agarose gel electrophoresis. LBP ORFs in successfully transformed clones are verified by automated dideoxy DNA sequencing. To transfect human kidney 293 cells, 1-2 μg of DNA is mixed with 6 μl lipofectamine reagent (Life Technologies) and incubated with the cells as described in the Life Technologies protocol. LBP expression in transfected cells is confirmed by Western blot analysis of cell extracts obtained 48 hr after transfection. To select for stably transfected 293 cells, the antibiotic G418 (Life Technologies) is added to the growth medium at a concentration of 800 μg/ml. Colonies resistant to G418 are tested for recombinant LBP expression by Western blot, and recombinant clones expressing LBPs are expanded, assayed for LDL binding and used to test compounds for their ability to inhibit LDL binding.

Example 18: In Vivo Screening for Agents Which Inhibit Binding Between LDL and LBPs

This example illustrates in vivo screening of agents which have been shown by in vitro tests to be promising candidate inhibitors of binding between LDL and LBPs.

In vivo inhibitory activity is first tested in the healing balloon-catheter deendothelialized rabbit aorta model of arterial injury (Roberts et al., J. Lipid Res. 24:1160-1167 (1983); Chang et al., Arterioscler. Thomb. 12:1088-1098 (1992)). This model was shown to be an excellent analog for human atherosclerotic lesions. Each candidate inhibitor is tested in five to ten ballooned rabbits, while an equal number of rabbits receive a control peptide, or placebo. Four weeks following aortic deendothelialization, when reendothelialization (healing) is partially complete, daily parenteral (intravenous or subcutaneous) or intragastric administration of the peptides and the analogs begins at an initial concentration of 10 mg/kg body weight, which is varied down, or up to 100 mg/kg depending on results. 30 min later, a bolus of intravenously injected ^{125}I (or ^{99m}Tc-) labeled LDL is given to test the candidate inhibitor's ability in short term studies to inhibit LDL sequestration in healing arterial lesions. If ^{125}I-LDL is used, the animals are sacrificed 8-24 hr later, the aortas excised, washed and subjected to quantitative autoradiography of excised aortas, as previously described (Roberts et al., J. Lipid Res. 24:1160-1167 (1983); Chang et al., Arterioscler. Thomb. 12:1088-1098 (1992)). If ^{99m}Tc-LDL is used,

analysis is by external gamma camera imaging of the live anesthetized animal at 2-24 hr, as previously described (Lees and Lees, Syndromes of Atherosclerosis, in Fuster, ed., Futura Publishing Co., Armonk, NY, pp. 385-401 (1996)), followed by sacrifice, excision and imaging of the excised aorta. Immediately before the end of testing, the animals have standard toxicity tests, including CBC, liver enzymes, and urinalysis.

The compounds which are most effective and least toxic are then tested in short term studies of rabbits fed a 2% cholesterol diet (Schwenke and Carew, Arteriosclerosis 9:895-907 (1989)). Each candidate inhibitor is tested in five to ten rabbits, while an equal number of rabbits receive a control peptide, or placebo. Animals receive one or more doses per day of the candidate inhibitor, or placebo, for up to two weeks. Daily frequency of doses is determined by route of administration. If active drug or placebo are administered parenterally, they are given 1-3 times daily and the 2% cholesterol diet is continued. If drug or placebo are given orally, they are mixed with the 2% cholesterol diet. Schwenke and Carew (Arteriosclerosis 9:895-907 (1989)) have shown that the LDL concentration in lesion-prone areas of the rabbit aorta is increased 22-fold above normal in rabbits fed a 2% cholesterol diet for 16 days and that the increased LDL content precedes the histological evidence of early atherosclerosis. Therefore, analysis of the effect of the candidate inhibitors is tested two weeks after the start of cholesterol feeding by injecting ^{125}I-LDL, allowing it to circulate for 8-24 hr, and then performing quantitative autoradiography on the excised aortas of both test and control animals. If appropriate, quantitation of aortic cholesterol content is also carried out (Schwenke and Carew, Arteriosclerosis 9:895-907 (1989); Schwenke and Carew, Arteriosclerosis 9:908-918 (1989).

The above procedures identify the most promising candidate inhibitors, as well as the best route and frequency of their administration. Inhibitors so identified are then tested in long-term studies of cholesterol-fed rabbits. These tests are carried out in the same way as the short-term cholesterol feeding studies, except that inhibitor effectiveness is tested by injection of ^{125}I-LDL at longer intervals following the initiation of cholesterol feeding, and lesion-prone areas of the aorta are examined histologically for evidence of atherosclerosis. Testing times are at two, four, and six months. Major arteries are examined grossly and histologically for evidence and extent of atherosclerosis. If necessary, other accepted animal models, such as atherosclerosis-susceptible primates (Williams et al., Arterioscler. Thromb. Vasc. Biol. 15:827-836 (1995) and/or Watanabe rabbits are tested with short- and long-term cholesterol feeding.

Example 19: In Vivo Inhibition of Radiolabeled LDL Accumulation in the Ballooned Deendothelialized Rabbit Aorta via Induction of Active Immunity Against LBP Protein

This example illustrates the effect that induction of immunity against LBP protein has on the accumulation of radiolabeled LDL in the ballooned deendothelialized rabbit aorta model of atherosclerosis.

Immunity was induced in male New Zealand White rabbits (Hazelton Research Products, Denver, PA) as follows: A mixture of purified human recombinant LBP-2 or BHF-1 peptide (1 ml; 1 mg) and RIBI adjuvant (RIBI ImmunoChem Research, Inc., Hamilton, MT) was injected subcutanously at 2-5 sites along the dorsal thoracic and abdominal regions of the rabbits. Blood was collected by venipuncture on days 1 (preimmune bleeding), 35, 63, and 91. Booster injections were administered on days 28 (500 μg; SC), 56 (250 μg; SC), and 84 (125 μg; SC).

The titer of the rabbits was evaluated by serial dilution using an ELISA plate format. Preimmune serum was evaluated at the same time. After the third booster of LBP protein or peptide, the titer reached a maximal level with a detectable colorimetric response on an ELISA plate of 156 pg. Titer is defined as the maximum dilution of antibody which generates an absorbance reading of 0.5 above control in 30 min. Specificity of the polyclonal antibodies was demonstrated using Western blot analysis as described in Example 6.

On day 93, the abdominal aorta of immunized and control rabbits was deendothelialized using a Fogarty number 4 embolectomy catheter (Chang et al., Arteriosclerosis and Thrombosis 12:1088-1098 (1992)). Four weeks after ballooning, rabbits received a bolus injection of ^{125}I-labeled LDL (1 ml; i.v.). Blood samples were collected at 1 hr intervals for 8 hr, and 24 hr post injection. Blood samples were centrifuged for 30 min at 2000 rpm (40°C) and total activity present in the serum was determined using a Gamma counter. Total TCA precipitable counts were determinined by addition of TCA to the serum to a final concentration of 10% followed by incubation for 10 min at 4°C. Serum samples were then centrifuged (2000 rpm; 30 min; 40°C) and total activity present in the supernate was determined. TCA precipitable counts were calculated by substration: total soluble counts minus counts present in the supernate after TCA precipitation. Blood samples for the determination of antibody titers were collected prior to the injection of the radiolabeled LDL.

After 24 hr, the rabbits were injected intravenously with 5% Evan's blue dye which was allowed to circulate for 15 min. Areas of the aorta in which the endothelial covering is absent stain blue while those areas covered by endothelium remain unstained. At the end of the

incubation period, the rabbits were euthanized and the abdominal and thoracic aorta were dissected out, rinsed, and fixed overnight in 10% TCA at room temperature. The aortas were then rinsed exhaustively with physiological saline, weighed, counted, blotted dry and placed onto X-ray film in order to visualize the pattern of radiolabeled LDL accumulation in the deendothelialized rabbit abdominal aorta.

Immunization of rabbits against recombinant human LBP-2 or BHF-1 peptide altered the pattern of radiolabeled LDL accumulation in the ballooned deendothelialized abdominal aorta. When corrected for dosage, and percent reendothelialization, immunized-ballooned rabbits had lower accumulation of radiolabeled LDL compared to nonimmune-ballooned rabbits. These results indicate that active immunization against LBP provides an effective means by which the accumulation of LDL in the injured arterial wall can be modified.

Example 20: Screening Agents in Humans Which Inhibit Binding Between LDL and LBPs

Human studies are carried out according to standard FDA protocols for testing of new drugs for safety (Phase I), efficacy (Phase II), and efficacy compared to other treatments (Phase III). Subjects, who are enrolled into studies after giving informed consent, are between the ages of 18 and 70. Women who are pregnant, or likely to become pregnant, or subjects with diseases other than primary atherosclerosis, such as cancer, liver disease, or diabetes, are excluded. Subjects selected for study in FDA Phase II and Phase III trials have atherosclerotic disease previously documented by standard techniques, such as ultrasound and/or angiography, or are known to be at high risk of atherosclerosis by virtue of having at least one first degree relative with documented atherosclerosis. Subjects themselves have normal or abnormal plasma lipids. Initial testing includes 20-50 subjects on active drug and 20-50 subjects, matched for age, sex, and atherosclerotic status, on placebo. The number of subjects is pre-determined by the number needed for statistical significance. Endpoints for inhibitor efficacy includes ultrasound measurements of carotid artery thickness in high risk subjects, as well as in subjects with known carotid or coronary disease; atherosclerotic events; atherosclerotic deaths; and all-cause deaths in all subjects. Non-invasive analysis (carotid artery thickness by ultrasound) as per Stadler (Med. and Biol. 22:25-34 (1996)) are carried out at 6- to 12-month intervals for 3 years. Atherosclerotic events and deaths, as well as all-cause deaths are tabulated at 3 years.

Oral dosage of drug in FDA Phase I trials ranges from 0.01 to 10 gm/day, and is

determined by results of animal studies, extrapolated on a per kg basis. Based on data obtained from Phase I studies, the dose range and frequency are narrowed in Phase II and III trials. If parenteral administration of drug is determined by animal studies to be the only effective method, parenteral administration in human subjects is tested by injection, as well as by the transdermal and nasal insufflation routes. Testing of parenteral drug follows the same outline as that for oral administration.

The optimal treatment schedule and dosage for humans is thus established.

Example 21: Treating an Individual Having Atherosclerosis with BHF-1

This example illustrates a method for treating an individual having atherosclerosis with an LBP fragment, e.g., BHF-1, so as to decrease the levels of arterially bound LDL in the individual. BHF-1 is obtained as described herein. The BHF-1 is administered to the mammal intravenously as a bolus or as an injection at a concentration of 0.5-10 mg/kg body weight. Such administrations are repeated indefinitely in order to prevent the development or progression of symptomatic atherosclerosis, just as is done currently with cholesterol-lowering drugs. Stable subjects are examined twice yearly to evaluate the extent of any atherosclerotic disease by physical exam and non-invasive studies, such as carotid artery thickness, ultrasound, and/or gamma camera imaging of the major arteries, to determine if atherosclerotic lesions are present, and, if previously present, have regressed or progressed. Such a regimen results in treatment of the atherosclerosis.

Those skilled in the art will be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lees, Ann M.
Lees, Robert S.
Law, Simon W.
Arjona, Anibal A.

(ii) TITLE OF INVENTION: NOVEL LOW DENSITY LIPOPROTEIN BINDING PROTEINS AND THEIR USE IN DIAGNOSING AND TREATING ATHEROSCLEROSIS

(iii) NUMBER OF SEQUENCES: 42

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Banner & Witcoff, Ltd.
(B) STREET: One Financial Center
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02111

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 and WordPerfect 6.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not available
(B) FILING DATE: November 26, 1997
(C) CLASSIFICATION: Not available

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Greer, Helen
(B) REGISTRATION NUMBER: 36,816
(C) REFERENCE/DOCKET NUMBER: 3983/59819

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-345-9100
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Ser Lys Asn Thr Val Ser Ser Ala Arg Phe Arg Lys Val Asp Val
1               5                  10                  15

Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp Gly Gly Asp
            20                  25                  30

Gly Gln Ala Gly Pro Asp Glu Gly Glu Val Asp Ser Cys Leu Arg Gln
        35                  40                  45

Gly Asn Met Thr Ala Ala Leu Gln Ala Ala Leu Lys Asn Pro Pro Ile
    50                  55                  60

Asn Thr Arg Ser Gln Ala Val Lys Asp Arg Ala Gly Ser Ile Val Leu
65                  70                  75                  80

Lys Val Leu Ile Ser Phe Lys Ala Gly Asp Ile Glu Lys Ala Val Gln
                85                  90                  95

Ser Leu Asp Arg Asn Gly Val Asp Leu Leu Met Lys Tyr Ile Tyr Lys
            100                 105                 110

Gly Phe Glu Ser Pro Ser Asp Asn Ser Ser Ala Val Leu Leu Gln Trp
        115                 120                 125

His Glu Lys Ala Leu Ala Ala Gly Gly Val Gly Ser Ile Val Arg Val
    130                 135                 140

Leu Thr Ala Arg Lys Thr Val
145                 150
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 317 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Asp Cys Arg Ser Ser Ser Asn Asn Arg Xaa Pro Lys Gly Gly Ala Ala
1               5                  10                  15

Arg Ala Gly Gly Pro Ala Arg Pro Val Ser Leu Arg Glu Val Val Arg
            20                  25                  30

Tyr Leu Gly Gly Ser Ser Gly Ala Gly Gly Arg Leu Thr Arg Gly Arg
        35                  40                  45
```

Val Gln Gly Leu Leu Glu Glu Glu Ala Ala Ala Arg Gly Arg Leu Glu
50 55 60

Arg Thr Arg Leu Gly Ala Leu Ala Leu Pro Arg Gly Asp Arg Pro Gly
65 70 75 80

Arg Ala Pro Pro Ala Ala Ser Ala Arg Ala Ala Arg Asn Lys Arg Ala
85 90 95

Gly Glu Glu Arg Val Leu Glu Lys Glu Glu Glu Glu Glu Glu Glu Glu
100 105 110

Asp Asp Glu Asp Asp Asp Asp Asp Val Val Ser Glu Gly Ser Glu Val
115 120 125

Pro Glu Ser Asp Arg Pro Ala Gly Ala Gln His His Gln Leu Asn Gly
130 135 140

Gly Glu Arg Gly Pro Gln Thr Ala Lys Glu Arg Ala Lys Glu Trp Ser
145 150 155 160

Leu Cys Gly Pro His Pro Gly Gln Glu Glu Gly Arg Gly Pro Ala Ala
165 170 175

Gly Ser Gly Thr Arg Gln Val Phe Ser Met Ala Ala Leu Ser Lys Glu
180 185 190

Gly Gly Ser Ala Ser Ser Thr Thr Gly Pro Asp Ser Pro Ser Pro Val
195 200 205

Pro Leu Pro Pro Gly Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro
210 215 220

Phe Gly Cys Pro Ala Gly Arg Lys Glu Lys Pro Ala Asp Pro Val Glu
225 230 235 240

Trp Thr Val Met Asp Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro
245 250 255

Glu Gln Ala Thr Ala Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu
260 265 270

Leu Leu Met Gln Arg Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu
275 280 285

Gly Pro Ala Leu Lys Ile Tyr Glu His His Ile Lys Val Leu Gln Gln
290 295 300

Gly His Phe Glu Asp Asp Asp Pro Glu Gly Phe Leu Gly
305 310 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Ala Ser Ala Arg Ala Ala Arg Asn Lys Arg Ala Gly Glu Glu Arg Val
1               5                  10                  15

Leu Glu Lys Glu Glu Glu Glu Glu Glu Glu Glu Asp Asp Glu Asp Asp
            20                  25                  30

Asp Asp Asp Val Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp Arg
        35                  40                  45

Pro Ala Gly Ala Gln His His Gln Leu Asn Gly Gly Glu Arg Gly Pro
    50                  55                  60

Gln Thr Ala Lys Glu Arg Ala Lys Glu Trp Ser Leu Cys Gly Pro His
65                  70                  75                  80

Pro Gly Gln Glu Glu Gly Arg Gly Pro Ala Ala Gly Ser Gly Thr Arg
                85                  90                  95

Gln Val Phe Ser Met Ala Ala Leu Ser Lys Glu Gly Gly Ser Ala Ser
            100                 105                 110

Ser Thr Thr Gly Pro Asp Ser Pro Ser Pro Val Pro Leu Pro Pro Gly
        115                 120                 125

Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro Phe Gly Cys Pro Ala
    130                 135                 140

Gly Arg Lys Glu Lys Pro Ala Asp Pro Val Glu Trp Thr Val Met Asp
145                 150                 155                 160

Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro Glu Gln Ala Thr Ala
                165                 170                 175

Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu Leu Leu Met Gln Arg
            180                 185                 190

Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu Gly Pro Ala Leu Lys
        195                 200                 205

Ile Tyr Glu His His Ile Lys Val Leu Gln Gln Gly His Phe Glu Asp
    210                 215                 220

Asp Asp Pro Glu Gly Phe Leu Gly
225                 230
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 252 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Thr Arg Leu Gly Ala Leu Ala Leu Pro Arg Gly Asp Arg Pro Gly Arg
1               5                  10                  15

Ala Pro Pro Ala Ala Ser Ala Arg Ala Ala Arg Asn Lys Arg Ala Gly
            20                  25                  30

Glu Glu Arg Val Leu Glu Lys Glu Glu Glu Glu Glu Glu Glu Glu Asp
        35                  40                  45

Asp Glu Asp Asp Asp Asp Asp Val Val Ser Glu Gly Ser Glu Val Pro
    50                  55                  60

Glu Ser Asp Arg Pro Ala Gly Ala Gln His His Gln Leu Asn Gly Gly
65                  70                  75                  80

Glu Arg Gly Pro Gln Thr Ala Lys Glu Arg Ala Lys Glu Trp Ser Leu
                85                  90                  95

Cys Gly Pro His Pro Gly Gln Glu Glu Gly Arg Gly Pro Ala Ala Gly
            100                 105                 110

Ser Gly Thr Arg Gln Val Phe Ser Met Ala Ala Leu Ser Lys Glu Gly
        115                 120                 125

Gly Ser Ala Ser Ser Thr Thr Gly Pro Asp Ser Pro Ser Pro Val Pro
    130                 135                 140

Leu Pro Pro Gly Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro Phe
145                 150                 155                 160

Gly Cys Pro Ala Gly Arg Lys Glu Lys Pro Ala Asp Pro Val Glu Trp
                165                 170                 175

Thr Val Met Asp Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro Glu
            180                 185                 190

Gln Ala Thr Ala Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu Leu
        195                 200                 205

Leu Met Gln Arg Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu Gly
    210                 215                 220
```

Pro Ala Leu Lys Ile Tyr Glu His His Ile Lys Val Leu Gln Gln Gly
225 230 235 240

His Phe Glu Asp Asp Asp Pro Glu Gly Phe Leu Gly
245 250

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 557 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Lys Asn Gln Asp Lys Lys Asn Gly Ala Ala Lys Gln Pro Asn Pro
1 5 10 15

Lys Ser Ser Pro Gly Gln Pro Glu Ala Gly Ala Glu Gly Ala Gln Gly
20 25 30

Arg Pro Gly Arg Pro Ala Pro Ala Arg Glu Ala Glu Gly Ala Ser Ser
35 40 45

Gln Ala Pro Gly Arg Pro Glu Gly Ala Gln Ala Lys Thr Ala Gln Pro
50 55 60

Gly Ala Leu Cys Asp Val Ser Glu Glu Leu Ser Arg Gln Leu Glu Asp
65 70 75 80

Ile Leu Ser Thr Tyr Cys Val Asp Asn Asn Gln Gly Ala Pro Gly Glu
85 90 95

Asp Gly Val Gln Gly Glu Pro Pro Glu Pro Glu Asp Ala Glu Lys Ser
100 105 110

Arg Ala Tyr Val Ala Arg Asn Gly Glu Pro Glu Pro Gly Thr Pro Val
115 120 125

Val Asn Gly Glu Lys Glu Thr Ser Lys Ala Glu Pro Gly Thr Glu Glu
130 135 140

Ile Arg Thr Ser Asp Glu Val Gly Asp Arg Asp His Arg Arg Pro Gln
145 150 155 160

Glu Lys Lys Lys Ala Lys Gly Leu Gly Lys Glu Ile Thr Leu Leu Met
165 170 175

Gln Thr Leu Asn Thr Leu Ser Thr Pro Glu Glu Lys Leu Ala Ala Leu
180 185 190

Cys Lys Lys Tyr Ala Glu Leu Leu Glu Glu His Arg Asn Ser Gln Lys
195 200 205

Gln Met Lys Leu Leu Gln Lys Lys Gln Ser Gln Leu Val Gln Glu Lys
210 215 220

Asp His Leu Arg Gly Glu His Ser Lys Ala Ile Leu Ala Arg Ser Lys
225 230 235 240

Leu Glu Ser Leu Cys Arg Glu Leu Gln Arg His Asn Arg Ser Leu Lys
245 250 255

Glu Glu Gly Val Gln Arg Ala Arg Glu Glu Glu Glu Lys Arg Lys Glu
260 265 270

Val Thr Ser His Phe Gln Met Thr Leu Asn Asp Ile Gln Leu Gln Met
275 280 285

Glu Gln His Asn Glu Arg Asn Ser Lys Leu Arg Gln Glu Asn Met Glu
290 295 300

Leu Ala Glu Arg Leu Lys Lys Leu Ile Glu Gln Tyr Glu Leu Arg Glu
305 310 315 320

Glu His Ile Asp Lys Val Phe Lys His Lys Asp Leu Gln Gln Gln Leu
325 330 335

Val Asp Ala Lys Leu Gln Gln Ala Gln Glu Met Leu Lys Glu Ala Glu
340 345 350

Glu Arg His Gln Arg Glu Lys Asp Phe Leu Leu Lys Glu Ala Val Glu
355 360 365

Ser Gln Arg Met Cys Glu Leu Met Lys Gln Gln Glu Thr His Leu Lys
370 375 380

Gln Gln Leu Ala Leu Tyr Thr Glu Lys Phe Glu Glu Phe Gln Asn Thr
385 390 395 400

Leu Ser Lys Ser Ser Glu Val Phe Thr Thr Phe Lys Gln Glu Met Glu
405 410 415

Lys Met Thr Lys Lys Ile Lys Lys Leu Glu Lys Glu Thr Thr Met Tyr
420 425 430

Arg Ser Arg Trp Glu Ser Ser Asn Lys Ala Leu Leu Glu Met Ala Glu
435 440 445

Glu Lys Thr Leu Arg Asp Lys Glu Leu Glu Gly Leu Gln Val Lys Ile
450 455 460

Gln Arg Leu Glu Lys Leu Cys Arg Ala Leu Gln Thr Glu Arg Asn Asp
465 470 475 480

Leu Asn Lys Arg Val Gln Asp Leu Ser Ala Gly Gly Gln Gly Pro Val
485 490 495

Ser Asp Ser Gly Pro Glu Arg Arg Pro Glu Pro Ala Thr Thr Ser Lys
500 505 510

Glu Gln Gly Val Glu Gly Pro Gly Ala Gln Val Pro Asn Ser Pro Arg
515 520 525

Ala Thr Asp Ala Ser Cys Cys Ala Gly Ala Pro Ser Thr Glu Ala Ser
530 535 540

Gly Gln Thr Gly Pro Gln Glu Pro Thr Thr Ala Thr Ala
545 550 555

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Lys Asn Thr Val Ser Ser Ala Arg Phe Arg Lys Val Asp Val
1 5 10 15

Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp Gly Gly Asp
20 25 30

Gly Gln Ala Gly Pro Asp Glu Gly Glu Val Asp Ser Cys Leu Arg Gln
35 40 45

Gly Asn Met Thr Ala Ala Leu Gln Ala Ala Leu Lys Asn Pro Pro Ile
50 55 60

Asn Thr Lys Ser Gln Ala Val Lys Asp Arg Ala Gly Ser Ile Val Leu
65 70 75 80

Lys Val Leu Ile Ser Phe Lys Ala Asn Asp Ile Glu Lys Ala Val Gln
85 90 95

Ser Leu Asp Lys Asn Gly Val Asp Leu Leu Met Lys Tyr Ile Tyr Lys
100 105 110

Gly Phe Glu Ser Pro Ser Asp Asn Ser Ser Ala Met Leu Leu Gln Trp
115 120 125

His Glu Lys Ala Leu Ala Ala Gly Gly Val Gly Ser Ile Val Arg Val
130 135 140

```
Leu Thr Ala Arg Lys Thr Val
145                 150
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 217 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Glu Glu Arg Val Leu Glu Lys Glu Glu Glu Glu Asp Asp Asp Glu Asp
1               5                  10                  15

Glu Asp Glu Glu Asp Asp Val Ser Glu Gly Ser Glu Val Pro Glu Ser
            20                  25                  30

Asp Arg Pro Ala Gly Ala Gln His His Gln Leu Asn Gly Glu Arg Gly
        35                  40                  45

Pro Gln Ser Ala Lys Glu Arg Val Lys Glu Trp Thr Pro Cys Gly Pro
    50                  55                  60

His Gln Gly Gln Asp Glu Gly Arg Gly Pro Ala Pro Gly Ser Gly Thr
65                  70                  75                  80

Arg Gln Val Phe Ser Met Ala Ala Met Asn Lys Glu Gly Gly Thr Ala
                85                  90                  95

Ser Val Ala Thr Gly Pro Asp Ser Pro Ser Pro Val Pro Leu Pro Pro
            100                 105                 110

Gly Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro Phe Gly Cys Pro
        115                 120                 125

Pro Gly Arg Lys Glu Lys Pro Ser Asp Pro Val Glu Trp Thr Val Met
    130                 135                 140

Asp Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro Glu Gln Ala Thr
145                 150                 155                 160

Ala Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu Leu Leu Met Gln
                165                 170                 175

Arg Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu Gly Pro Ala Leu
            180                 185                 190

Lys Ile Tyr Glu His His Ile Lys Val Leu Gln Gln Gly His Phe Glu
        195                 200                 205
```

Asp Asp Asp Pro Asp Gly Phe Leu Gly
210 215

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 530 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Ser Ser Pro Gly Gln Pro Glu Ala Gly Pro Glu Gly Ala Gln Glu
1 5 10 15

Arg Pro Ser Gln Ala Ala Pro Ala Val Glu Ala Glu Gly Pro Gly Ser
20 25 30

Ser Gln Ala Pro Arg Lys Pro Glu Gly Ala Gln Ala Arg Thr Ala Gln
35 40 45

Ser Gly Ala Leu Arg Asp Val Ser Glu Glu Leu Ser Arg Gln Leu Glu
50 55 60

Asp Ile Leu Ser Thr Tyr Cys Val Asp Asn Asn Gln Gly Gly Pro Gly
65 70 75 80

Glu Asp Gly Ala Gln Gly Glu Pro Ala Glu Pro Glu Asp Ala Glu Lys
85 90 95

Ser Arg Thr Tyr Val Ala Arg Asn Gly Glu Pro Glu Pro Thr Pro Val
100 105 110

Val Tyr Gly Glu Lys Glu Pro Ser Lys Gly Asp Pro Asn Thr Glu Glu
115 120 125

Ile Arg Gln Ser Asp Glu Val Gly Asp Arg Asp His Arg Arg Pro Gln
130 135 140

Glu Lys Lys Lys Ala Lys Gly Leu Gly Lys Glu Ile Thr Leu Leu Met
145 150 155 160

Gln Thr Leu Asn Thr Leu Ser Thr Pro Glu Glu Lys Leu Ala Ala Leu
165 170 175

Cys Lys Lys Tyr Ala Glu Leu Leu Glu Glu His Arg Asn Ser Gln Lys
180 185 190

Gln Met Lys Leu Leu Gln Lys Lys Gln Ser Gln Leu Val Gln Glu Lys
195 200 205

Asp His Leu Arg Gly Glu His Ser Lys Ala Val Leu Ala Arg Ser Lys
210 215 220

Leu Glu Ser Leu Cys Arg Glu Leu Gln Arg His Asn Arg Ser Leu Lys
225 230 235 240

Glu Glu Gly Val Gln Arg Ala Arg Glu Glu Glu Glu Lys Arg Lys Glu
245 250 255

Val Thr Ser His Phe Gln Val Thr Leu Asn Asp Ile Gln Leu Gln Met
260 265 270

Glu Gln His Asn Glu Arg Asn Ser Lys Leu Arg Gln Glu Asn Met Glu
275 280 285

Leu Ala Glu Arg Leu Lys Lys Leu Ile Glu Gln Tyr Glu Leu Arg Glu
290 295 300

Glu His Ile Asp Lys Val Phe Lys His Lys Asp Leu Gln Gln Gln Leu
305 310 315 320

Val Asp Ala Lys Leu Gln Gln Ala Gln Glu Met Leu Lys Glu Ala Glu
325 330 335

Glu Arg His Gln Arg Glu Lys Asp Phe Leu Leu Lys Glu Ala Val Glu
340 345 350

Ser Gln Arg Met Cys Glu Leu Met Lys Gln Gln Glu Thr His Leu Lys
355 360 365

Gln Gln Leu Ala Leu Tyr Thr Glu Lys Phe Glu Glu Phe Gln Asn Thr
370 375 380

Leu Ser Lys Ser Ser Glu Val Phe Thr Thr Phe Lys Gln Glu Met Glu
385 390 395 400

Lys Met Thr Lys Lys Ile Lys Lys Leu Glu Lys Glu Thr Thr Met Tyr
405 410 415

Arg Ser Arg Trp Glu Ser Ser Asn Lys Ala Leu Leu Glu Met Ala Glu
420 425 430

Glu Lys Thr Val Arg Asp Lys Glu Leu Glu Gly Leu Gln Val Lys Ile
435 440 445

Gln Arg Leu Glu Lys Leu Cys Arg Ala Leu Gln Thr Glu Arg Asn Asp
450 455 460

Leu Asn Lys Arg Val Gln Asp Leu Ser Ala Gly Gly Gln Gly Ser Leu
465 470 475 480

Thr Asp Ser Gly Pro Glu Arg Arg Pro Glu Gly Pro Gly Ala Gln Ala
485 490 495

```
Pro Ser Ser Pro Arg Val Thr Glu Ala Pro Cys Tyr Pro Gly Ala Pro
            500                 505                 510

Ser Thr Glu Ala Ser Gly Gln Thr Gly Pro Gln Glu Pro Thr Ser Ala
        515                 520                 525

Arg Ala
    530
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Val Asp Val Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp
1               5                  10                  15

Gly Gly Asp Gly
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1404 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
AAGCCTCGCA GCGGTCGGGG CGGCGCCGCG GAGGCTCGAG GGCGGCGGGC GGCGGCG          57

ATG TCG AAG AAC ACG GTG TCG TCG GCG CGG TTC CGG AAG GTG GAC GTG         105
Met Ser Lys Asn Thr Val Ser Ser Ala Arg Phe Arg Lys Val Asp Val
  1               5                  10                  15

GAT GAG TAC GAC GAG AAC AAG TTC GTG GAC GAG GAA GAC GGC GGC GAC         153
Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp Gly Gly Asp
            20                  25                  30

GGC CAG GCG GGG CCG GAC GAG GGC GAG GTG GAC TCG TGC CTG CGG CAA         201
Gly Gln Ala Gly Pro Asp Glu Gly Glu Val Asp Ser Cys Leu Arg Gln
        35                  40                  45

GGG AAC ATG ACA GCC GCC CTG CAG GCG GCG CTG AAG AAC CCT CCC ATC         249
Gly Asn Met Thr Ala Ala Leu Gln Ala Ala Leu Lys Asn Pro Pro Ile
    50                  55                  60
```

```
AAC ACC AGG AGC CAG GCG GTG AAG GAC CGG GCA GGC AGC ATC GTG CTG        297
Asn Thr Arg Ser Gln Ala Val Lys Asp Arg Ala Gly Ser Ile Val Leu
 65                  70                  75                  80

AAG GTG CTC ATC TCC TTC AAG GCC GGC GAC ATA GAA AAG GCC GTG CAG        345
Lys Val Leu Ile Ser Phe Lys Ala Gly Asp Ile Glu Lys Ala Val Gln
                 85                  90                  95

TCC CTG GAC AGG AAC GGC GTG GAC CTG CTC ATG AAG TAC ATC TAC AAG        393
Ser Leu Asp Arg Asn Gly Val Asp Leu Leu Met Lys Tyr Ile Tyr Lys
            100                 105                 110

GGC TTC GAG AGC CCC TCC GAC AAC AGC AGC GCC GTG CTC CTG CAG TGG        441
Gly Phe Glu Ser Pro Ser Asp Asn Ser Ser Ala Val Leu Leu Gln Trp
        115                 120                 125

CAC GAG AAG GCG CTG GCT GCA GGA GGA GTG GGC TCC ATC GTC CGT GTC        489
His Glu Lys Ala Leu Ala Ala Gly Gly Val Gly Ser Ile Val Arg Val
    130                 135                 140

CTG ACT GCA AGG AAA ACC GTG TAGCCTGGCA GGAACGGGTG CCTGCCGGGG           540
Leu Thr Ala Arg Lys Thr Val
145                 150
AGCGGGAGCT GCCGGTACAA AGACCAAAAC GCCCAGATGC CGCCGCTGCC CTGTGGGCGG      600

CGTCTGTTCC CAGCTTCGCT TTTTCCCTTT CCCGTGTCTG TCAGGATTAC ATAAGGTTTC      660

CCTTCGTGAG AATCGGAGTG GCGCAGAGGG TCCTGTTCAT ACGCGCCGTG CGTCCGGCTG      720

TGTAAGACCC CTGCCTTCAG TGTCCTTGAG CAACGGTAGC GTGTCGCCGG CTGGGTTTGG      780

TTTTGTCGTG GAGGGATCTG GTCAGAATTT GAGGCCAGTT TCCTAACTCA TTGCTGGTCA      840

GGAAATGATC TTCATTTAAA AAAAAAAAAA AGACTGGCAG CTATTATGCA AAACTGGACC      900

CTCTTCCCTT ATTTAAGCAG AGTGAGTTTC TGGAACCAGT GGTGCCCCCC CCCCCGCCCC      960

GGCCGCCGTC CTGCTCAAGG GAAGCCTCCC TGCAGAGCAG CAGAGCCCCT GGGCAGGAGC     1020

GCCGCGTCCC GCTCCCAGGA GACAGCATGC GCGGTCACGC GGCACTTCCT GTGCCTCCCA     1080

GCCCCAGTGC CCCGGAGTTC TTCAGGGCGA CAGGGACCTC AGAAGACTGG ATCCGATCCA     1140

GACAGACGCC CATTCTTGGT TCAGCTCAGT GTTTTCAAAA GGAACGTGCT ACCGTGGGTA     1200

GAGCACACTG GTTCTCAGAA CACGGCCGGC GCTTGACGGT TGTCACAGCT CCAGAACAAA     1260

TCCTGGGAGA CAGGCGAGCG CGAGTCGCCG GGCAGGAATT CCACACACTC GTGCTGTTTT     1320

TGATACCTGC TTTTTGTTTT GTTTTGTAAA AATGATGCAC TTGAGAAAAT AAAACGTCAG     1380

TGTTGACAAA AAAAAAAAAA AAAA                                            1404
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1617 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GAC TGC CGC AGC AGC AGC AAC AAC CGC TAG CCG AAG GGT GGC GCG GCG          48
Asp Cys Arg Ser Ser Ser Asn Asn Arg Xaa Pro Lys Gly Gly Ala Ala
 1               5                  10                  15

CGG GCC GGC GGC CCG GCG CGG CCC GTG AGC CTG CGG GAA GTC GTG CGC          96
Arg Ala Gly Gly Pro Ala Arg Pro Val Ser Leu Arg Glu Val Val Arg
            20                  25                  30

TAC CTC GGG GGT AGC AGC GGC GCT GGC GGC CGC CTG ACC CGC GGC CGC         144
Tyr Leu Gly Gly Ser Ser Gly Ala Gly Gly Arg Leu Thr Arg Gly Arg
        35                  40                  45

GTG CAG GGT CTG CTG GAA GAG GAG GCG GCG GCG CGG GGC CGC CTG GAG         192
Val Gln Gly Leu Leu Glu Glu Glu Ala Ala Ala Arg Gly Arg Leu Glu
    50                  55                  60

CGC ACC CGT CTC GGA GCG CTT GCG CTG CCC CGC GGG GAC AGG CCC GGA         240
Arg Thr Arg Leu Gly Ala Leu Ala Leu Pro Arg Gly Asp Arg Pro Gly
65                  70                  75                  80

CGG GCG CCA CCG GCC GCC AGC GCC CGC GCG GCG CGG AAC AAG AGA GCT         288
Arg Ala Pro Pro Ala Ala Ser Ala Arg Ala Ala Arg Asn Lys Arg Ala
                85                  90                  95

GGC GAG GAG CGA GTG CTT GAA AAG GAG GAG GAG GAG GAG GAG GAG GAA         336
Gly Glu Glu Arg Val Leu Glu Lys Glu Glu Glu Glu Glu Glu Glu Glu
            100                 105                 110

GAC GAC GAG GAC GAC GAC GAC GAC GTC GTG TCC GAG GGC TCG GAG GTG         384
Asp Asp Glu Asp Asp Asp Asp Asp Val Val Ser Glu Gly Ser Glu Val
        115                 120                 125

CCC GAG AGC GAT CGT CCC GCG GGT GCG CAG CAT CAC CAG CTG AAT GGC         432
Pro Glu Ser Asp Arg Pro Ala Gly Ala Gln His His Gln Leu Asn Gly
    130                 135                 140

GGC GAG CGC GGC CCG CAG ACC GCC AAG GAG CGG GCC AAG GAG TGG TCG         480
Gly Glu Arg Gly Pro Gln Thr Ala Lys Glu Arg Ala Lys Glu Trp Ser
145                 150                 155                 160

CTG TGT GGC CCC CAC CCT GGC CAG GAG GAA GGG CGG GGG CCG GCC GCG         528
Leu Cys Gly Pro His Pro Gly Gln Glu Glu Gly Arg Gly Pro Ala Ala
                165                 170                 175

GGC AGT GGC ACC CGC CAG GTG TTC TCC ATG GCG GCC TTG AGT AAG GAG         576
Gly Ser Gly Thr Arg Gln Val Phe Ser Met Ala Ala Leu Ser Lys Glu
            180                 185                 190

GGG GGA TCA GCC TCT TCG ACC ACC GGG CCT GAC TCC CCG TCC CCG GTG         624
Gly Gly Ser Ala Ser Ser Thr Thr Gly Pro Asp Ser Pro Ser Pro Val
        195                 200                 205
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CCT TTG CCC CCC GGG AAG CCA GCC CTC CCA GGA GCC GAT GGG ACC CCC        672
Pro Leu Pro Pro Gly Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro
    210                 215                 220

TTT GGC TGC CCT GCC GGG CGC AAA GAG AAG CCG GCA GAC CCC GTG GAG        720
Phe Gly Cys Pro Ala Gly Arg Lys Glu Lys Pro Ala Asp Pro Val Glu
225                 230                 235                 240

TGG ACA GTC ATG GAC GTC GTG GAG TAC TTC ACC GAG GCG GGC TTC CCT        768
Trp Thr Val Met Asp Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro
                245                 250                 255

GAG CAA GCC ACG GCT TTC CAG GAG CAG GAG ATC GAC GGC AAG TCC CTG        816
Glu Gln Ala Thr Ala Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu
            260                 265                 270

CTG CTC ATG CAG CGC ACC GAT GTC CTC ACC GGC CTG TCC ATC CGC CTG        864
Leu Leu Met Gln Arg Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu
        275                 280                 285

GGG CCA GCG TTG AAA ATC TAT GAG CAC CAT ATC AAG GTG CTG CAG CAG        912
Gly Pro Ala Leu Lys Ile Tyr Glu His His Ile Lys Val Leu Gln Gln
    290                 295                 300

GGT CAC TTC GAG GAC GAT GAC CCG GAA GGC TTC CTG GGA TGAGCACAGA         961
Gly His Phe Glu Asp Asp Asp Pro Glu Gly Phe Leu Gly
305                 310                 315

GCCGCCGCGC CCCTTGTCCC CACCCCCACC CCGCCTGGAC CCATTCCTGC CTCCATGTCA    1021

CCCAAGGTGT CCCAGAGGCC AGGAGCTGGA CTGGGCAGGC GAGGGGTGCG GACCTACCCT    1081

GATTCTGGTA GGGGGCGGGG CCTTGCTGTG CTCATTGCTA CCCCCCCACC CCGTGTGTGT    1141

CTCTGCACCT GCCCCCAGCA CACCCCTCCC GGAGCCTGGA TGTCGCCTGG GACTCTGGCC    1201

TGCTCATTTT GCCCCCAGAT CAGCCCCCTC CCTCCCTCCT GTCCCAGGAC ATTTTTTAAA    1261

AGAAAAAAAG GAAAAAAAAA AATTGGGGAG GGGGCTGGGA AGGTGCCCCA AGATCCTCCT    1321

CGGCCCAACC AGGTGTTTAT TCCTATATAT ATATATATAT GTTTTGTTCT GCCTGTTTTT    1381

CGTTTTTTGG TGCGTGGCCT TTCTTCCCTC CCACCACCAC TCATGGCCCC AGCCCTGCTC    1441

GCCCTGTCGG CGGGAGCAGC TGGGAATGGG AGGAGGGTGG GACCTTGGGT CTGTCTCCCA    1501

CCCTCTCTCC CGTTGGTTCT GTTGTCGCTC CAGCTGGCTG TATTGCTTTT TAATATTGCA    1561

CCGAAGGGTT GTTTTTTTTT TTTTAAATAA AATTTTAAAA AAAGGAAAAA AAAAAA        1617
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1362 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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GCC AGC CCC CGC GCG GCG CGG AAC AAG AGA GCT GGC GAG GAG CGA GTG          48
Ala Ser Ala Arg Ala Ala Arg Asn Lys Arg Ala Gly Glu Glu Arg Val
1               5                   10                  15

CTT GAA AAG GAG GAG GAG GAG GAG GAG GAG GAA GAC GAC GAG GAC GAC          96
Leu Glu Lys Glu Glu Glu Glu Glu Glu Glu Glu Asp Asp Glu Asp Asp
            20                  25                  30

GAC GAC GAC GTC GTG TCC GAG GGC TCG GAG GTG CCC GAG AGC GAT CGT         144
Asp Asp Asp Val Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp Arg
        35                  40                  45

CCC GCG GGT GCG CAG CAT CAC CAG CTG AAT GGC GGC GAG CGC GGC CCG         192
Pro Ala Gly Ala Gln His His Gln Leu Asn Gly Gly Glu Arg Gly Pro
    50                  55                  60

CAG ACC GCC AAG GAG CGG GCC AAG GAG TGG TCG CTG TGT GGC CCC CAC         240
Gln Thr Ala Lys Glu Arg Ala Lys Glu Trp Ser Leu Cys Gly Pro His
65                  70                  75                  80

CCT GGC CAG GAG GAA GGG CGG GGG CCG GCC GCG GGC AGT GGC ACC CGC         288
Pro Gly Gln Glu Glu Gly Arg Gly Pro Ala Ala Gly Ser Gly Thr Arg
                85                  90                  95

CAG GTG TTC TCC ATG GCG GCC TTG AGT AAG GAG GGG GGA TCA GCC TCT         336
Gln Val Phe Ser Met Ala Ala Leu Ser Lys Glu Gly Gly Ser Ala Ser
            100                 105                 110

TCG ACC ACC GGG CCT GAC TCC CCG TCC CCG GTG CCT TTG CCC CCC GGG         384
Ser Thr Thr Gly Pro Asp Ser Pro Ser Pro Val Pro Leu Pro Pro Gly
        115                 120                 125

AAG CCA GCC CTC CCA GGA GCC GAT GGG ACC CCC TTT GGC TGC CCT GCC         432
Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro Phe Gly Cys Pro Ala
    130                 135                 140

GGG CGC AAA GAG AAG CCG GCA GAC CCC GTG GAG TGG ACA GTC ATG GAC         480
Gly Arg Lys Glu Lys Pro Ala Asp Pro Val Glu Trp Thr Val Met Asp
145                 150                 155                 160

GTC GTG GAG TAC TTC ACC GAG GCG GGC TTC CCT GAG CAA GCC ACG GCT         528
Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro Glu Gln Ala Thr Ala
                165                 170                 175

TTC CAG GAG CAG GAG ATC GAC GGC AAG TCC CTG CTG CTC ATG CAG CGC         576
Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu Leu Leu Met Gln Arg
            180                 185                 190

ACC GAT GTC CTC ACC GGC CTG TCC ATC CGC CTG GGG CCA GCG TTG AAA         624
Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu Gly Pro Ala Leu Lys
        195                 200                 205
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```
ATC TAT GAG CAC CAT ATC AAG GTG CTG CAG CAG GGT CAC TTC GAG GAC        672
Ile Tyr Glu His His Ile Lys Val Leu Gln Gln Gly His Phe Glu Asp
    210                 215                 220

GAT GAC CCG GAA GGC TTC CTG GGA TGAGCACAGA GCCGCCGCGC CCCTTGTCCC        726
Asp Asp Pro Glu Gly Phe Leu Gly
225                 230

CACCCCCACC CCGCCTGGAC CCATTCCTGC CTCCATGTCA CCCAAGGTGT CCCAGAGGCC 786

AGGAGCTGGA CTGGGCAGGC GAGGGGTGCG GACCTACCCT GATTCTGGTA GGGGGCGGGG 846

CCTTGCTGTG CTCATTGCTA CCCCCCCACC CCGTGTGTGT CTCTGCACCT GCCCCCAGCA 906

CACCCCTCCC GGAGCCTGGA TGTCGCCTGG GACTCTGGCC TGCTCATTTT GCCCCCAGAT 966

CAGCCCCCTC CCTCCCTCCT GTCCCAGGAC ATTTTTTAAA AGAAAAAAAG GAAAAAAAAA 1026

AATTGGGGAG GGGGCTGGGA AGGTGCCCCA AGATCCTCCT CGGCCCAACC AGGTGTTTAT 1086

TCCTATATAT ATATATATAT GTTTTGTTCT GCCTGTTTTT CGTTTTTTGG TGCGTGGCCT 1146

TTCTTCCCTC CCACCACCAC TCATGGCCCC AGCCCTGCTC GCCCTGTCGG CGGGAGCAGC 1206

TGGGAATGGG AGGAGGGTGG GACCTTGGGT CTGTCTCCCA CCCTCTCTCC CGTTGGTTCT 1266

GTTGTCGCTC CAGCTGGCTG TATTGCTTTT TAATATTGCA CCGAAGGGTT GTTTTTTTTT 1326

TTTTAAATAA AATTTTAAAA AAAGGAAAAA AAAAAA                          1362
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1422 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
ACC CGT CTC GGA GCG CTT GCG CTG CCC CGC GGG GAC AGG CCC GGA CGG         48
Thr Arg Leu Gly Ala Leu Ala Leu Pro Arg Gly Asp Arg Pro Gly Arg
1               5                   10                  15

GCG CCA CCG GCC GCC AGC GCC CGC GCG GCG CGG AAC AAG AGA GCT GGC         96
Ala Pro Pro Ala Ala Ser Ala Arg Ala Ala Arg Asn Lys Arg Ala Gly
            20                  25                  30

GAG GAG CGA GTG CTT GAA AAG GAG GAG GAG GAG GAG GAG GAG GAA GAC        144
Glu Glu Arg Val Leu Glu Lys Glu Glu Glu Glu Glu Glu Glu Glu Asp
        35                  40                  45

GAC GAG GAC GAC GAC GAC GAC GTC GTG TCC GAG GGC TCG GAG GTG CCC        192
Asp Glu Asp Asp Asp Asp Asp Val Val Ser Glu Gly Ser Glu Val Pro
    50                  55                  60
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GAG AGC GAT CGT CCC GCG GGT GCG CAG CAT CAC CAG CTG AAT GGC GGC          240
Glu Ser Asp Arg Pro Ala Gly Ala Gln His His Gln Leu Asn Gly Gly
65                  70                  75                  80

GAG CGC GGC CCG CAG ACC GCC AAG GAG CGG GCC AAG GAG TGG TCG CTG          288
Glu Arg Gly Pro Gln Thr Ala Lys Glu Arg Ala Lys Glu Trp Ser Leu
                85                  90                  95

TGT GGC CCC CAC CCT GGC CAG GAG GAA GGG CGG GGG CCG GCC GCG GGC          336
Cys Gly Pro His Pro Gly Gln Glu Glu Gly Arg Gly Pro Ala Ala Gly
            100                 105                 110

AGT GGC ACC CGC CAG GTG TTC TCC ATG GCG GCC TTG AGT AAG GAG GGG          384
Ser Gly Thr Arg Gln Val Phe Ser Met Ala Ala Leu Ser Lys Glu Gly
        115                 120                 125

GGA TCA GCC TCT TCG ACC ACC GGG CCT GAC TCC CCG TCC CCG GTG CCT          432
Gly Ser Ala Ser Ser Thr Thr Gly Pro Asp Ser Pro Ser Pro Val Pro
    130                 135                 140

TTG CCC CCC GGG AAG CCA GCC CTC CCA GGA GCC GAT GGG ACC CCC TTT          480
Leu Pro Pro Gly Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro Phe
145                 150                 155                 160

GGC TGC CCT GCC GGG CGC AAA GAG AAG CCG GCA GAC CCC GTG GAG TGG          528
Gly Cys Pro Ala Gly Arg Lys Glu Lys Pro Ala Asp Pro Val Glu Trp
                165                 170                 175

ACA GTC ATG GAC GTC GTG GAG TAC TTC ACC GAG GCG GGC TTC CCT GAG          576
Thr Val Met Asp Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro Glu
            180                 185                 190

CAA GCC ACG GCT TTC CAG GAG CAG GAG ATC GAC GGC AAG TCC CTG CTG          624
Gln Ala Thr Ala Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu Leu
        195                 200                 205

CTC ATG CAG CGC ACC GAT GTC CTC ACC GGC CTG TCC ATC CGC CTG GGG          672
Leu Met Gln Arg Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu Gly
    210                 215                 220

CCA GCG TTG AAA ATC TAT GAG CAC CAT ATC AAG GTG CTG CAG CAG GGT          720
Pro Ala Leu Lys Ile Tyr Glu His His Ile Lys Val Leu Gln Gln Gly
225                 230                 235                 240

CAC TTC GAG GAC GAT GAC CCG GAA GGC TTC CTG GGA TGAGCACAGA               766
His Phe Glu Asp Asp Asp Pro Glu Gly Phe Leu Gly
                245                 250

GCCGCCGCGC CCCTTGTCCC CACCCCCACC CCGCCTGGAC CCATTCCTGC CTCCATGTCA 826

CCCAAGGTGT CCCAGAGGCC AGGAGCTGGA CTGGGCAGGC GAGGGGTGCG GACCTACCCT 886

GATTCTGGTA GGGGGCGGGG CCTTGCTGTG CTCATTGCTA CCCCCCCACC CCGTGTGTGT 946

CTCTGCACCT GCCCCCAGCA CACCCCTCCC GGAGCCTGGA TGTCGCCTGG GACTCTGGCC 1006

TGCTCATTTT GCCCCCAGAT CAGCCCCCTC CCTCCCTCCT GTCCCAGGAC ATTTTTTAAA 1066
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AGAAAAAAAG GAAAAAAAAA AATTGGGGAG GGGGCTGGGA AGGTGCCCCA AGATCCTCCT 1126

CGGCCCAACC AGGTGTTTAT TCCTATATAT ATATATATAT GTTTTGTTCT GCCTGTTTTT 1186

CGTTTTTTGG TGCGTGGCCT TTCTTCCCTC CCACCACCAC TCATGGCCCC AGCCCTGCTC 1246

GCCCTGTCGG CGGGAGCAGC TGGGAATGGG AGGAGGGTGG GACCTTGGGT CTGTCTCCCA 1306

CCCTCTCTCC CGTTGGTTCT GTTGTCGCTC CAGCTGGCTG TATTGCTTTT TAATATTGCA 1366

CCGAAGGGTT GTTTTTTTTT TTTTAAATAA AATTTTAAAA AAAGGAAAAA AAAAAA      1422
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4722 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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GTGGAAAATA GCAACTGTGT TTCTCAAGGA TCCAATCCCA ACCTAAGGTG GCAGCGCACA       60

ATG AAG AAT CAA GAC AAA AAG AAC GGG GCT GCC AAA CAG CCC AAC CCC         108
Met Lys Asn Gln Asp Lys Lys Asn Gly Ala Ala Lys Gln Pro Asn Pro
  1               5                  10                  15

AAA AGC AGC CCG GGA CAG CCG GAA GCA GGA GCG GAG GGA GCC CAG GGG         156
Lys Ser Ser Pro Gly Gln Pro Glu Ala Gly Ala Glu Gly Ala Gln Gly
             20                  25                  30

CGG CCC GGC CGG CCG GCC CCC GCC CGA GAA GCC GAA GGT GCC AGC AGC         204
Arg Pro Gly Arg Pro Ala Pro Ala Arg Glu Ala Glu Gly Ala Ser Ser
         35                  40                  45

CAG GCT CCC GGG AGG CCG GAG GGG GCT CAA GCC AAA ACT GCT CAG CCT         252
Gln Ala Pro Gly Arg Pro Glu Gly Ala Gln Ala Lys Thr Ala Gln Pro
     50                  55                  60

GGG GCG CTC TGT GAT GTC TCT GAG GAG CTG AGC CGC CAG TTG GAA GAC         300
Gly Ala Leu Cys Asp Val Ser Glu Glu Leu Ser Arg Gln Leu Glu Asp
 65                  70                  75                  80

ATA CTC AGT ACA TAC TGT GTG GAC AAC AAC CAG GGG GCC CCG GGT GAG         348
Ile Leu Ser Thr Tyr Cys Val Asp Asn Asn Gln Gly Ala Pro Gly Glu
                 85                  90                  95

GAT GGG GTC CAG GGT GAG CCC CCT GAA CCT GAA GAT GCA GAG AAG TCT         396
Asp Gly Val Gln Gly Glu Pro Pro Glu Pro Glu Asp Ala Glu Lys Ser
            100                 105                 110

CGC GCC TAT GTG GCA AGG AAT GGG GAG CCG GAG CCG GGC ACC CCA GTA         444
Arg Ala Tyr Val Ala Arg Asn Gly Glu Pro Glu Pro Gly Thr Pro Val
        115                 120                 125
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GTC AAT GGC GAG AAG GAG ACC TCC AAG GCA GAG CCG GGC ACG GAA GAG          492
Val Asn Gly Glu Lys Glu Thr Ser Lys Ala Glu Pro Gly Thr Glu Glu
    130                 135                 140

ATC CGG ACG AGC GAT GAG GTC GGA GAC CGA GAC CAC CGG AGG CCA CAG          540
Ile Arg Thr Ser Asp Glu Val Gly Asp Arg Asp His Arg Arg Pro Gln
145                 150                 155                 160

GAA AAG AAG AAG GCC AAG GGT CTG GGA AAG GAG ATC ACG CTG CTG ATG          588
Glu Lys Lys Lys Ala Lys Gly Leu Gly Lys Glu Ile Thr Leu Leu Met
                165                 170                 175

CAG ACA CTG AAC ACG CTG AGC ACC CCA GAG GAG AAG CTG GCG GCT CTG          636
Gln Thr Leu Asn Thr Leu Ser Thr Pro Glu Glu Lys Leu Ala Ala Leu
            180                 185                 190

TGC AAG AAG TAT GCG GAA CTG CTC GAG GAG CAC CGG AAC TCG CAG AAG          684
Cys Lys Lys Tyr Ala Glu Leu Leu Glu Glu His Arg Asn Ser Gln Lys
        195                 200                 205

CAG ATG AAG CTG CTG CAG AAG AAG CAG AGC CAG CTG GTG CAG GAG AAG          732
Gln Met Lys Leu Leu Gln Lys Lys Gln Ser Gln Leu Val Gln Glu Lys
    210                 215                 220

GAC CAC CTG CGT GGC GAG CAC AGC AAG GCC ATC CTG GCC CGC AGC AAG          780
Asp His Leu Arg Gly Glu His Ser Lys Ala Ile Leu Ala Arg Ser Lys
225                 230                 235                 240

CTC GAG AGC CTG TGC CGG GAG CTG CAG CGG CAC AAC CGC TCG CTC AAG          828
Leu Glu Ser Leu Cys Arg Glu Leu Gln Arg His Asn Arg Ser Leu Lys
                245                 250                 255

GAA GAA GGT GTG CAG CGA GCC CGA GAG GAG GAG GAG AAG CGC AAG GAG          876
Glu Glu Gly Val Gln Arg Ala Arg Glu Glu Glu Glu Lys Arg Lys Glu
            260                 265                 270

GTG ACG TCA CAC TTC CAG ATG ACG CTC AAC GAC ATT CAG CTG CAG ATG          924
Val Thr Ser His Phe Gln Met Thr Leu Asn Asp Ile Gln Leu Gln Met
        275                 280                 285

GAG CAG CAC AAC GAG CGC AAC TCC AAG CTG CGC CAG GAG AAC ATG GAG          972
Glu Gln His Asn Glu Arg Asn Ser Lys Leu Arg Gln Glu Asn Met Glu
    290                 295                 300

CTG GCC GAG CGG CTC AAG AAG CTG ATT GAG CAG TAC GAG CTG CGA GAA         1020
Leu Ala Glu Arg Leu Lys Lys Leu Ile Glu Gln Tyr Glu Leu Arg Glu
305                 310                 315                 320

GAG CAC ATC GAC AAA GTC TTC AAA CAC AAG GAT CTG CAG CAG CAG CTG         1068
Glu His Ile Asp Lys Val Phe Lys His Lys Asp Leu Gln Gln Gln Leu
                325                 330                 335

GTG GAC GCC AAG CTC CAG CAG GCC CAG GAG ATG CTG AAG GAG GCA GAG         1116
Val Asp Ala Lys Leu Gln Gln Ala Gln Glu Met Leu Lys Glu Ala Glu
            340                 345                 350
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GAG CGG CAC CAG CGG GAG AAG GAC TTT CTC CTG AAG GAG GCC GTG GAG      1164
Glu Arg His Gln Arg Glu Lys Asp Phe Leu Leu Lys Glu Ala Val Glu
        355                 360                 365

TCC CAG AGG ATG TGC GAG CTG ATG AAG CAA CAG GAG ACC CAC CTG AAG      1212
Ser Gln Arg Met Cys Glu Leu Met Lys Gln Gln Glu Thr His Leu Lys
    370                 375                 380

CAG CAG CTT GCC CTA TAC ACA GAG AAG TTT GAG GAG TTC CAG AAC ACT      1260
Gln Gln Leu Ala Leu Tyr Thr Glu Lys Phe Glu Glu Phe Gln Asn Thr
385                 390                 395                 400

CTT TCC AAA AGC AGC GAG GTG TTC ACC ACA TTC AAA CAG GAA ATG GAA      1308
Leu Ser Lys Ser Ser Glu Val Phe Thr Thr Phe Lys Gln Glu Met Glu
                405                 410                 415

AAG ATG ACA AAG AAG ATC AAG AAG CTG GAG AAA GAG ACC ACC ATG TAC      1356
Lys Met Thr Lys Lys Ile Lys Lys Leu Glu Lys Glu Thr Thr Met Tyr
            420                 425                 430

CGT TCC CGG TGG GAG AGC AGC AAC AAG GCC CTG CTT GAG ATG GCT GAG      1404
Arg Ser Arg Trp Glu Ser Ser Asn Lys Ala Leu Leu Glu Met Ala Glu
        435                 440                 445

GAG AAA ACA CTC CGG GAC AAA GAG CTG GAA GGC CTG CAG GTG AAA ATC      1452
Glu Lys Thr Leu Arg Asp Lys Glu Leu Glu Gly Leu Gln Val Lys Ile
    450                 455                 460

CAG CGG CTG GAG AAG CTG TGC CGG GCA CTG CAG ACA GAG CGC AAT GAC      1500
Gln Arg Leu Glu Lys Leu Cys Arg Ala Leu Gln Thr Glu Arg Asn Asp
465                 470                 475                 480

CTG AAC AAG AGG GTG CAG GAC CTG AGT GCC GGT GGC CAG GGC CCC GTC      1548
Leu Asn Lys Arg Val Gln Asp Leu Ser Ala Gly Gly Gln Gly Pro Val
                485                 490                 495

TCC GAC AGC GGT CCT GAG CGG AGG CCA GAG CCC GCC ACC ACC TCC AAG      1596
Ser Asp Ser Gly Pro Glu Arg Arg Pro Glu Pro Ala Thr Thr Ser Lys
            500                 505                 510

GAG CAG GGT GTC GAG GGC CCC GGG GCT CAA GTA CCC AAC TCT CCA AGG      1644
Glu Gln Gly Val Glu Gly Pro Gly Ala Gln Val Pro Asn Ser Pro Arg
        515                 520                 525

GCC ACA GAC GCT TCC TGC TGC GCA GGT GCA CCC AGC ACA GAG GCA TCA      1692
Ala Thr Asp Ala Ser Cys Cys Ala Gly Ala Pro Ser Thr Glu Ala Ser
    530                 535                 540

GGC CAG ACA GGG CCC CAG GAG CCC ACC ACT GCC ACT GCC TAGAGAGCTT       1741
Gly Gln Thr Gly Pro Gln Glu Pro Thr Thr Ala Thr Ala
545                 550                 555

GGTGCTGGGG TGTGCCAGGA AGGGAGCAGG CAGCCCAGCC AGGCCTGGCC CAGCCCAGGC    1801

TCCCATGCTA AGCAGTCCGG TGCTGAGGCC AGGATGTTCT GACCTGGCTG GCACCTGACC    1861

CTCTGCAGTC TTGGATTTTG TGGGTCAGTT TTACATGCAT ATGGCACACA TGCAAGGCCT    1921
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CACACATTTG TGTCTCTAAG TGTACTGTGG GCTTGCATCG GGGGTGACGA TGGACAGATG 1981

AAGCCAGCGG CTCCCTTGTG AGCTGAAGTC TTACGGAGGA GACGGCGTCT GCACTGCCAT 2041

CGCAGTGACC TGCAGGACGA GTTCCTTGAG CTTTCCCTGC CTGCTTTGAG GCTGAGACCC 2101

CTCCCGGCCC TTCAGAGCTC CTGACAGGTG ATACACACCC AGCCTTGACC GCACTTCTCT 2161

TGGGTAGCTG GGCTCTCCTA GCCTCCCCCA GAGGCGCCAT TGCTTCTCTT GACTTGGAGA 2221

GGGGATGCCC AGGCGTGGCC TTGGCAGGCA CTGGGAGCTA GTGATTGGGC TGCTCTCCTG 2281

CCTCGAGCAG GGGCAGGAGT GTTTCTGGTG GGATGATGCG CTCGCTGGTC AGGAGCCCCG 2341

TGGGCGCTGC TTCCCCCGCC CTCTGGTGAT GCCAGGACCA GGCCAGTGAT GCTTCTCAGT 2401

AGCCTTACCA TTCACAGGTG CCTCTCCAGC CCGCACAGTG AGTGACAAGA TCATCCAAAG 2461

GATTCCTTCT GAAGGTGTTC GTTTCGTTTT GTTTTGTTGC ACGTGACGGT TTGTATTGAG 2521

GACCCTCTGA GGAAGAGGGG TGCTGTAGCA GTGGTCCCTG CGTGCCTGGC TCCAGTGTCC 2581

TGCCCTCCCC CCCCTCGCCA TGGCTCCTCG GCCGCCTTGG TGCTGAGGTT TCTGTTTGGT 2641

GAGATCAGGT TGTCTGTTCA GAGAGAAGAG GCGTCTGATG GCTTTGCCGC CAGCTTGCCT 2701

GCGGGCCTCA ATCCCGGGAG GCCGCCCGGT TCCCGTCACT GTTGTCCCCG TGCAGTGCGT 2761

TGCTGGTCCC CAGGACCAGC TGCTCGTTTG CTGTATGGGT CAGTTTCTGC TTCCTGCCCC 2821

CCACTCCACC TAACTGCAAT CCTTGGGGTT TCCCTGGTTC TCGTCCCTGG TACCTCTGTG 2881

CCCAAGAAGT AGCCTTCTTT GGGATTCTTG TTCTGCCCAT GCGGGAGCTG CTGCTGTCTG 2941

ACAGGTGAGG CCTGAGACTC AGCGGCTGAC AGAGCTGCAG AGCTCTGCAC GGTGGCTCCC 3001

GGGGCGGCCT CTGTGTGCTG CACACCGCTG CTCTGCTGGC ACTGGCCAGT CTGTGCAGAG 3061

CATTTGAGTA CTGGCTCAGG AGGGAGGGCT CTGCTGGCCT CGAGGGACAG CGCCACGTCT 3121

CCAGCTGGGC TCAGGGAGAG CCCCAGACTG GCTGCGTAGG GTGCTTGGGG TTTGCTTCTT 3181

GCAGTATTTC TTGGAAGCTG TTTTGTTGTC CTGCTATTCC TTCATCTTCC ACAGTCCACG 3241

CTCAGCCTTT AACTTGGATC CCTCACATAA CAGGGTTCAT GAGACCCGCA AGTACGCCCA 3301

AGCTACGTAT GGCTGAGGCC AGCTGGCAGG TGAATGGCAC GCCATTGCTG CTGCTAATCC 3361

CTGGCATATC TTTAGTTCAC CTCGAAATGC CCCCGCCACA GTGCAAGCAG TGAGTCCACG 3421

TGCCACCCTG GGCTGAATCC CACCCCCTGT GAGTGTTGCC CGAGATTGTG TCTCTTCTGA 3481

ATGCCTTCAC TGGGAATGGC CTCTGCCGCC TCCTGCTCAG GGAGGCTTTC CCCTTCCCTC 3541

AGCCCCTGTG CCAGACTGAG GTACAAGAAC CGCCAAGCCC ATGCAAGGTG TGGCTAGGCG 3601

CCAGGGTGCA GGAAGGAGGC AGGTAGCTGC CTGCACCCTT GAAAGCCAAG AGGCCTACGG 3661

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TGGCCTCCAT CCTGGCTTGC CTCACTTCAG CTACCTCGCA TAGCCCAGGG GTGGGGCTAT    3721

TGGATTCCAG GGTGGGGGGA TGGGAAGCTG CAGGGGGCAG GTGGCTCTCA CTAGGCTTCC    3781

CAGCTCAGGA ATGTGGGCCT CAGGTAGGGG AGAGCCTTTG CTCCACTCCA CCCATTTGCA    3841

GGCATCTAGG CCAGTCTAGA TGGCGACCCC TTCTCTTCCT CTCCATTGAC CAAATCGTAC    3901

CTGTCTCTCC AGCTGCTCGC TTGCTCTGCT TTCCAAAGTC AGCCCAGGTA CCCAGGTGCC    3961

GCCCACATTG GCCTGGAACC TGGACCAGAG GCAAGGGAGG TGGCCTATCC TTGAGTGATA    4021

GCCAGTGCCT TCCTCACCCG GTGGCTTCCA TGCCTGTGAC CTCAGATTTA GGACCAAGAG    4081

CTGTGTTGGT TTCTTACGTT GTGAGCTTTC CCTCCAGGGG ACCACAGCAG GTGAGGCTCG    4141

GAGCCCAGAG CCCTTGGCGC CGCCAGCAGT AACTTGTGTC CGGACCTTGT CCAGCTGAGC    4201

GCTTCGTGTA TGACTCAGCT TCGTGTGTGA GTCCAGCGGA GTGCGTCACG TGACCTAGAC    4261

TCAGCGGTGT CAGCCGCACT TTGATTTGTT TGTTTTCCAT GAGGTTTTTG GACCATGGGC    4321

TTAGCTCAGG CAACTTTTCT GTAAGGAGAA TGTTAACTTT CTGTAAAGAT GCTTATTTAA    4381

CTAACGCCTG CTTCCCCCAC TCCCAACCAG GTGGCCACCG AGAGCTCACC AGGAGGCCAA    4441

TAGAGCTGCT CCAGCTCTCC CATCTTGCAC CGCACAAAGG TGGCCGCCCC AGGGACAGCC    4501

AGGCACCTGC CTGGGGGAGG GGCTTCTCTT CCTTATGGCC TGGCCATCTA GATTGTTTAA    4561

AGTTGTGCTG ACAGCTTTTT TTGGTTTTTT GGTTTTTGTT TTTGTTTTTG TTTTTGTTTT    4621

TGTCTACTTT TGGTATTCAC AACAGCCAGG GACTTGATTT TGATGTATTT TAAGCCACAT    4681

TAAATAAAGA GTCTGTTGCC TTAAAAAAAA AAAAAAAAAA A                        4722
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1928 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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GACGCCTCAG AGCGGAACAG GGAAGTGAAT CAGGCGCCGG GTAGTGGGTT GCTGGGCTGG      60

GCTTGCTGAG GTAGAGGCAG CGCCAAGAAG AGGCCTTTGC CGCTGGTCGG GATTGGG        117

ATG TCG AAG AAC ACA GTG TCG TCG GCC CGC TTC CGG AAG GTG GAC GTG        165
Met Ser Lys Asn Thr Val Ser Ser Ala Arg Phe Arg Lys Val Asp Val
  1               5                  10                  15
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GAT GAA TAT GAC GAG AAC AAG TTC GTG GAC GAA GAA GAT GGG GGC GAC          213
Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp Gly Gly Asp
             20                  25                  30

GGC CAG GCC GGG CCC GAC GAG GGC GAG GTG GAC TCC TGC CTG CGG CAA          261
Gly Gln Ala Gly Pro Asp Glu Gly Glu Val Asp Ser Cys Leu Arg Gln
         35                  40                  45

GGA AAC ATG ACA GCT GCC CTA CAG GCA GCT CTG AAG AAC CCC CCT ATC          309
Gly Asn Met Thr Ala Ala Leu Gln Ala Ala Leu Lys Asn Pro Pro Ile
     50                  55                  60

AAC ACC AAG AGT CAG GCA GTG AAG GAC CGG GCA GGC AGC ATT GTC TTG          357
Asn Thr Lys Ser Gln Ala Val Lys Asp Arg Ala Gly Ser Ile Val Leu
 65                  70                  75                  80

AAG GTG CTC ATC TCT TTT AAA GCT AAT GAT ATA GAA AAG GCA GTT CAA          405
Lys Val Leu Ile Ser Phe Lys Ala Asn Asp Ile Glu Lys Ala Val Gln
                 85                  90                  95

TCT CTG GAC AAG AAT GGT GTG GAT CTC CTA ATG AAG TAT ATT TAT AAA          453
Ser Leu Asp Lys Asn Gly Val Asp Leu Leu Met Lys Tyr Ile Tyr Lys
            100                 105                 110

GGA TTT GAG AGC CCG TCT GAC AAT AGC AGT GCT ATG TTA CTG CAA TGG          501
Gly Phe Glu Ser Pro Ser Asp Asn Ser Ser Ala Met Leu Leu Gln Trp
        115                 120                 125

CAT GAA AAG GCA CTT GCT GCT GGA GGA GTA GGG TCC ATT GTT CGT GTC          549
His Glu Lys Ala Leu Ala Ala Gly Gly Val Gly Ser Ile Val Arg Val
    130                 135                 140

TTG ACT GCA AGA AAA ACT GTG TAGTCTGGCA GGAAGTGGAT TATCTGCCTC             600
Leu Thr Ala Arg Lys Thr Val
145                 150

GGGAGTGGGA ATTGCTGGTA CAAAGACCAA AACAACCAAA TGCCACCGCT GCCCTGTGGG        660

TAGCATCTGT TTCTCTCAGC TTTGCCTTCT TGCTTTTTCA TATCTGTAAA GAAAAAAATT        720

ACATATCAGT TGTCCCTTTA ATGAAAATTG GGATAATATA GAAGAAATTG TGTTAAAATA        780

GAAGTGTTTC ATCCTTTCAA AACCATTTCA GTGATGTTTA TACCAATCTG TATATAGTAT        840

AATTTACATT CAAGTTTTAA TTGTGCAACT TTTAACCCTG TTGGCTGGTT TTTGGTTCTG        900

TTTGGTTTTG TATTATTTTT AACTAATACT GAAAAATTTG GTCAGAATTT GAGGCCAGTT        960

TCCTAGCTCA TTGCTAGTCA GGAAATGATA TTTATAAAAA ATATGAGAGA CTGGCAGCTA       1020

TTAACATTGC AAAACTGGAC CATATTTCCC TTATTTAATA AGCAAAATAT GTTTTTGGAA       1080

TAAGTGGTGG GTGAATACCA CTGCTAAGTT ATAGCTTTGT TTTTGCTTGC CTCCTCATTA       1140

TCTGTACTGT GGGTTTAAGT ATGCTACTTT CTCTCAGCAT CCAATAATCA TGGCCCCTCA       1200

ATTTATTTGT GGTCACGCAG GGTTCAGAGC AAGAAGTCTT GCTTTATACA AATGTATCCA       1260
```

```
TAAAATATCA GAGCTTGTTG GGCATGAACA TCAAACTTTT GTTCCACTAA TATGGCTCTG    1320

TTTGGAAAAA ACTGCAAATC AGAAAGAATG ATTTGCAGAA AGAAAGAAAA ACTATGGTGT    1380

AATTTAAACT CTGGGCAGCC TCTGAATGAA ATGCTACTTT CTTTAGAAAT ATAATAGCTG    1440

CCTTAGACAT TATGAGGTAT ACAACTAGTA TTTAAGATAC CATTTAATAT GCCCCGTAAA    1500

TGTCTTCAGT GTTCTTCAGG GTAGTTGGGA TCTCAAAAGA TTTGGTTCAG ATCCAAACAA    1560

ATACACATTC TGTGTTTTAG CTCAGTGTTT TCTAAAAAAA GAAACTGCCA CACAGCAAAA    1620

AATTGTTTAC TTTGTTGGAC AAACCAAATC AGTTCTCAAA AAATGACCGG TGCTTATAAA    1680

AAGTTATAAA TATCGAGTAG CTCTAAAACA AACCACCTGA CCAAGAGGGA AGTGAGCTTG    1740

TGCTTAGTAT TTACATTGGA TGCCAGTTTT GTAATCACTG ACTTATGTGC AAACTGGTGC    1800

AGAAATTCTA TAAACTCTTT GCTGTTTTTG ATACCTGCTT TTTGTTTCAT TTTGTTTTGT    1860

TTTGTAAAAA TGATAAAACT TCAGAAAATA AAATGTCAGT GTTGAATAAT TAAAAAAAAA    1920

AAAAA                                                                1925
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1207 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GAA GAG CGA GTA CTT GAG AAA GAA GAG GAA GAA GAT GAT GAT GAA GAT        48
Glu Glu Arg Val Leu Glu Lys Glu Glu Glu Glu Asp Asp Asp Glu Asp
  1               5                  10                  15

GAA GAT GAA GAA GAT GAT GTG TCA GAG GGC TCT GAA GTG CCC GAG AGT        96
Glu Asp Glu Glu Asp Asp Val Ser Glu Gly Ser Glu Val Pro Glu Ser
            20                  25                  30

GAC CGT CCT GCA GGT GCC CAG CAC CAC CAG CTT AAC GGC GAG CGG GGA       144
Asp Arg Pro Ala Gly Ala Gln His His Gln Leu Asn Gly Glu Arg Gly
        35                  40                  45

CCT CAG AGT GCC AAG GAG AGG GTC AAG GAG TGG ACC CCC TGC GGA CCG       192
Pro Gln Ser Ala Lys Glu Arg Val Lys Glu Trp Thr Pro Cys Gly Pro
    50                  55                  60

CAC CAG GGC CAG GAT GAA GGG CGG GGG CCA GCC CCG GGC AGC GGC ACC       240
His Gln Gly Gln Asp Glu Gly Arg Gly Pro Ala Pro Gly Ser Gly Thr
65                  70                  75                  80
```

```
CGC CAG GTG TTC TCC ATG GCA GCC ATG AAC AAG GAA GGG GGA ACA GCT        288
Arg Gln Val Phe Ser Met Ala Ala Met Asn Lys Glu Gly Gly Thr Ala
                85                  90                  95

TCT GTT GCC ACC GGG CCA GAC TCC CCG TCC CCC GTG CCT TTG CCC CCA        336
Ser Val Ala Thr Gly Pro Asp Ser Pro Ser Pro Val Pro Leu Pro Pro
            100                 105                 110

GGC AAA CCA GCC CTA CCT GGG GCC GAC GGG ACC CCC TTT GGC TGT CCT        384
Gly Lys Pro Ala Leu Pro Gly Ala Asp Gly Thr Pro Phe Gly Cys Pro
        115                 120                 125

CCC GGG CGC AAA GAG AAG CCA TCT GAT CCC GTC GAG TGG ACC GTG ATG        432
Pro Gly Arg Lys Glu Lys Pro Ser Asp Pro Val Glu Trp Thr Val Met
    130                 135                 140

GAT GTC GTC GAA TAT TTT ACT GAG GCT GGA TTC CCG GAG CAG GCG ACA        480
Asp Val Val Glu Tyr Phe Thr Glu Ala Gly Phe Pro Glu Gln Ala Thr
145                 150                 155                 160

GCT TTC CAA GAG CAG GAA ATT GAT GGC AAA TCT TTG CTG CTC ATG CAG        528
Ala Phe Gln Glu Gln Glu Ile Asp Gly Lys Ser Leu Leu Leu Met Gln
                165                 170                 175

CGC ACA GAT GTG CTC ACC GGC CTG TCC ATC CGC CTC GGG CCA GCC CTG        576
Arg Thr Asp Val Leu Thr Gly Leu Ser Ile Arg Leu Gly Pro Ala Leu
            180                 185                 190

AAA ATC TAC GAG CAC CAC ATC AAG GTG CTT CAG CAA GGC CAC TTT GAG        624
Lys Ile Tyr Glu His His Ile Lys Val Leu Gln Gln Gly His Phe Glu
        195                 200                 205

GAT GAT GAC CCC GAT GGC TTC TTA GGC TGAGCGCCCA GCCTCACCCC              671
Asp Asp Asp Pro Asp Gly Phe Leu Gly
    210                 215

TGCCCCAGCC CATTCCGGCC CCCATCTCAC CCAAGATCCC CCAGAGTCCA GGAGCTGGAC      731

GGGGACACCC TCAGCCCTCA TAACAGATTC CAAGGAGAGG GCACCCTCTT GTCCTTATCT      791

TTGCCCCTTG TGTCTGTCTC ACACACATCT GCTCCTCAGC ACGTCGGTGT GGGGAGGGGA      851

TTGCTCCTTA AACCCCAGGT GGCTGACCCT CCCCACCCAG TCCAGGACAT TTTAGGAAAA      911

AAAAAATGAA ATGTGGGGGG CTTCTCATCT CCCCAAGATC CTCTTCCGTT CAGCCAGATG      971

TTTCCTGTAT AAATGTTTGG ATCTGCCTGT TTATTTTGGT GGGTGGTCTT TCCTCCCTCC     1031

CCTACCACCC ATGCCCCCCT TCTCAGTCTG CCCCTGGCCT CCAGCCCCTA GGGGACTAGC     1091

TGGGTTGGGG TTCCTCGGGC CTTTTCTCTC CTCCCTCTTT TCTTTCTGTT GATTGTCGCT     1151

CCAGCTGGCT GTATTGCTTT TTAATATTGC ACCGAAGGTT TTTTAAATAA AATTTTA        1208
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4697 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
CA AAA AGC AGC CCA GGA CAA CCG GAA GCA GGA CCC GAG GGA GCC CAG          47
   Lys Ser Ser Pro Gly Gln Pro Glu Ala Gly Pro Glu Gly Ala Gln
   1               5                  10                  15

GAG CGG CCC AGC CAG GCG GCT CCT GCA GTA GAA GCA GAA GGT CCC GGC          95
Glu Arg Pro Ser Gln Ala Ala Pro Ala Val Glu Ala Glu Gly Pro Gly
                20                  25                  30

AGC AGC CAG GCT CCT CGG AAG CCG GAG GGG GCT CAA GCC AGA ACG GCT         143
Ser Ser Gln Ala Pro Arg Lys Pro Glu Gly Ala Gln Ala Arg Thr Ala
            35                  40                  45

CAG TCT GGG GCC CTT CGT GAT GTC TCT GAG GAG CTG AGC CGC CAA CTG         191
Gln Ser Gly Ala Leu Arg Asp Val Ser Glu Glu Leu Ser Arg Gln Leu
        50                  55                  60

GAA GAC ATA CTG AGC ACA TAC TGT GTG GAC AAT AAC CAG GGG GGC CCC         239
Glu Asp Ile Leu Ser Thr Tyr Cys Val Asp Asn Asn Gln Gly Gly Pro
    65                  70                  75

GGC GAG GAT GGG GCA CAG GGT GAG CCG GCT GAA CCC GAA GAT GCA GAG         287
Gly Glu Asp Gly Ala Gln Gly Glu Pro Ala Glu Pro Glu Asp Ala Glu
80                  85                  90                  95

AAG TCC CGG ACC TAT GTG GCA AGG AAT GGG GAG CCT GAA CCA ACT CCA         335
Lys Ser Arg Thr Tyr Val Ala Arg Asn Gly Glu Pro Glu Pro Thr Pro
                100                 105                 110

GTA GTC TAT GGA GAG AAG GAA CCC TCC AAG GGG GAT CCA AAC ACA GAA         383
Val Val Tyr Gly Glu Lys Glu Pro Ser Lys Gly Asp Pro Asn Thr Glu
            115                 120                 125

GAG ATC CGG CAG AGT GAC GAG GTC GGA GAC CGA GAC CAT CGA AGG CCA         431
Glu Ile Arg Gln Ser Asp Glu Val Gly Asp Arg Asp His Arg Arg Pro
        130                 135                 140

CAG GAG AAG AAA AAA GCC AAG GGT TTG GGG AAG GAG ATC ACG TTG CTG         479
Gln Glu Lys Lys Lys Ala Lys Gly Leu Gly Lys Glu Ile Thr Leu Leu
    145                 150                 155

ATG CAG ACA TTG AAT ACT CTG AGT ACC CCA GAG GAG AAG CTG GCT GCT         527
Met Gln Thr Leu Asn Thr Leu Ser Thr Pro Glu Glu Lys Leu Ala Ala
160                 165                 170                 175

CTG TGC AAG AAG TAT GCT GAA CTG CTG GAG GAG CAC CGG AAT TCA CAG         575
Leu Cys Lys Lys Tyr Ala Glu Leu Leu Glu Glu His Arg Asn Ser Gln
                180                 185                 190
```

```
AAG CAG ATG AAG CTC CTA CAG AAA AAG CAG AGC CAG CTG GTG CAA GAG          623
Lys Gln Met Lys Leu Leu Gln Lys Lys Gln Ser Gln Leu Val Gln Glu
            195                 200                 205

AAG GAC CAC CTG CGC GGT GAG CAC AGC AAG GCC GTC CTG GCC CGC ACC          671
Lys Asp His Leu Arg Gly Glu His Ser Lys Ala Val Leu Ala Arg Ser
        210                 215                 220

AAG CTT GAG AGC CTA TGC CGT GAG CTG CAG CGG CAC AAC CGC TCC CTC          719
Lys Leu Glu Ser Leu Cys Arg Glu Leu Gln Arg His Asn Arg Ser Leu
    225                 230                 235

AAG GAA GAA GGT GTG CAG CGG GCC CGG GAG GAG GAG GAG AAG CGC AAG          767
Lys Glu Glu Gly Val Gln Arg Ala Arg Glu Glu Glu Glu Lys Arg Lys
240                 245                 250                 255

GAG GTG ACC TCG CAC TTC CAG GTG ACA CTG AAT GAC ATT CAG CTG CAG          815
Glu Val Thr Ser His Phe Gln Val Thr Leu Asn Asp Ile Gln Leu Gln
                260                 265                 270

ATG GAA CAG CAC AAT GAG CGC AAC TCC AAG CTG CGC CAA GAG AAC ATG          863
Met Glu Gln His Asn Glu Arg Asn Ser Lys Leu Arg Gln Glu Asn Met
            275                 280                 285

GAG CTG GCT GAG AGG CTC AAG AAG CTG ATT GAG CAG TAT GAG CTG CGC          911
Glu Leu Ala Glu Arg Leu Lys Lys Leu Ile Glu Gln Tyr Glu Leu Arg
        290                 295                 300

GAG GAG CAT ATC GAC AAA GTC TTC AAA CAC AAG GAC CTA CAA CAG CAG          959
Glu Glu His Ile Asp Lys Val Phe Lys His Lys Asp Leu Gln Gln Gln
    305                 310                 315

CTG GTG GAT GCC AAG CTC CAG CAG GCC CAG GAG ATG CTA AAG GAG GCA         1007
Leu Val Asp Ala Lys Leu Gln Gln Ala Gln Glu Met Leu Lys Glu Ala
320                 325                 330                 335

GAA GAG CGG CAC CAG CGG GAG AAG GAT TTT CTC CTG AAA GAG GCA GTA         1055
Glu Glu Arg His Gln Arg Glu Lys Asp Phe Leu Leu Lys Glu Ala Val
                340                 345                 350

GAG TCC CAG AGG ATG TGT GAG CTG ATG AAG CAG CAA GAG ACC CAC CTG         1103
Glu Ser Gln Arg Met Cys Glu Leu Met Lys Gln Gln Glu Thr His Leu
            355                 360                 365

AAG CAA CAG CTT GCC CTA TAC ACA GAG AAG TTT GAG GAG TTC CAG AAC         1151
Lys Gln Gln Leu Ala Leu Tyr Thr Glu Lys Phe Glu Glu Phe Gln Asn
        370                 375                 380

ACA CTT TCC AAA AGC AGC GAG GTA TTC ACC ACA TTC AAG CAG GAG ATG         1199
Thr Leu Ser Lys Ser Ser Glu Val Phe Thr Thr Phe Lys Gln Glu Met
    385                 390                 395

GAA AAG ATG ACT AAG AAG ATC AAG AAG CTG GAG AAA GAA ACC ACC ATG         1247
Glu Lys Met Thr Lys Lys Ile Lys Lys Leu Glu Lys Glu Thr Thr Met
400                 405                 410                 415
```

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TAC CGG TCC CGG TGG GAG AGC AGC AAC AAG GCC CTG CTT GAG ATG GCT          1295
Tyr Arg Ser Arg Trp Glu Ser Ser Asn Lys Ala Leu Leu Glu Met Ala
                420                 425                 430

GAG GAG AAA ACA GTC CGG GAT AAA GAA CTG GAG GGC CTG CAG GTA AAA          1343
Glu Glu Lys Thr Val Arg Asp Lys Glu Leu Glu Gly Leu Gln Val Lys
            435                 440                 445

ATC CAA CGG CTG GAG AAG CTG TGC CGG GCA CTG CAG ACA GAG CGC AAT          1391
Ile Gln Arg Leu Glu Lys Leu Cys Arg Ala Leu Gln Thr Glu Arg Asn
        450                 455                 460

GAC CTG AAC AAG AGG GTA CAG GAC CTG AGT GCT GGT GGC CAG GGC TCC          1439
Asp Leu Asn Lys Arg Val Gln Asp Leu Ser Ala Gly Gly Gln Gly Ser
    465                 470                 475

CTC ACT GAC AGT GGC CCT GAG AGG AGG CCA GAG GGG CCT GGG GCT CAA          1487
Leu Thr Asp Ser Gly Pro Glu Arg Arg Pro Glu Gly Pro Gly Ala Gln
480                 485                 490                 495

GCA CCC AGC TCC CCC AGG GTC ACA GAA GCG CCT TGC TAC CCA GGA GCA          1535
Ala Pro Ser Ser Pro Arg Val Thr Glu Ala Pro Cys Tyr Pro Gly Ala
                500                 505                 510

CCG AGC ACA GAA GCA TCA GGC CAG ACT GGG CCT CAA GAG CCC ACC TCC          1583
Pro Ser Thr Glu Ala Ser Gly Gln Thr Gly Pro Gln Glu Pro Thr Ser
            515                 520                 525

GCC AGG GCC TAGAGAGCCT GGTGTTGGGT CATGCTGGGA AGGGAGCGGC AGCCCAGCCA      1642
Ala Arg Ala
        530

GGCCTGGCCC ATAAAAGGCT CCCATGCTGA GCAGCCCATT GCTGAAGCCA GGATGTTCTT       1702

GACCTGGCTG GCATCTGGCA CTTGCAATTT TGGATTTTGT GGGTCAGTTT TACGTACATA       1762

GGGCATTTTG CAAGGCCTTG CAAATGCATT TATACCTGTA AGTGTACAGT GGGCTTGCAT       1822

TGGGGATGGG GGTGTGTACA GATGAAGTCA GTGGCTTGTC TGTGAGCTGA AGAGTCTTGA       1882

GAGGGGCTGT CATCTGTAGC TGCCATCACA GTGAGTTGGC AGAAGTGACT TGAGCATTTC       1942

TCTGTCTGAT TTGAGGCTCA GACCCCTCCC TGCCCTTTCA GAGCTCAAAA CAAGTAATAC       2002

ACCAAGGTCT TGACTGCATT TGTCTTGTGA GCAGGGCTTG CTTGGTCAGC TCAGGCCCTC       2062

CTAGCTGCTT GGAGGCTCCT TTGATTCTCT AGACCTGGAA AAGGTGTCCC TAGGCAGAGC       2122

CCTGGCAGGG CGCTCAGAGC TGGGATTTCC TGCCTGGAAC AAGGGACCTG GAGAATGTTT       2182

TTGCGTGGGA TGATGTGCTG GTCAGGAGCC CCTTGGGCAT CGCTTCCCCT GCCCTTTGGT       2242

AGTGCCAGGA CCAGGCCAAT GATGCTTCTC AGTAGCCTTA TCATTCACAG GTGCCTCTCT       2302

AGCCTGCACA AATGATTGAC AAGAGATCAC CCAAAGGATT ATTTCTGAAG GTGTTTTTTT       2362

CTTTATTTCT TTTTCTTTTT TTTTTTTTCT TTTTCTTTTT TTTTTGCACA TGACAGTGTT       2422
```

TGTATTGAGG ACCTTCCAAG GAAAAGGGAT GCTGTACCAG TGGTGCCTGG GTGCCTGGCC 2482

TCCAGTGTCC CACCTCCTTC ACCACCCCAC TTGGCTCCTT TGCCATCTTG ATGCTGAGGT 2542

TTCCTGTTTG GTGAGATCAG GTTGTTTGTG GTAAAAGAAA GGAAAGGGCT TCTGATGGCT 2602

TTGCCACAAG CTTACCTGTG GGTTTCAGTC CTGAGAGGCC ACCACCAGTT CCCATCAGCA 2662

CTGTCTCCAT GCAGCAGTTG CTGGGTCCCA TGTCCAGCTG CCTCTTTGGC TTCATGGGTT 2722

TTTCTGCTTC CTGCCCCCAC CCCCACATGT GCAATCCTCA AGATTTGTCC TGATTCTATT 2782

TCCTGGCACC TCCCTGCCTG TCCTTGGGGA TTCTACTTCT TCCTGTGTGG GGCCCATAGC 2842

TGTTGTCTAA CAGGTAAGAA ATGAAATTGA ACTATTGACT GGGCCCCAGA AATCCATAAA 2902

ATGGCTGCAG ACAGTTGTTT CTGTGTCCTG TTCTACCCCC ACTCCAGTAC ATAACTACTA 2962

TGTACTGTGT AGAGCCATTC TATATGCTGA ATGTTCTGCT GTTGCAAACT TGCCAGGGTA 3022

TTAGCCAGTG TTTGTGCCAA GCAGTTTTCG GGGACAACAG AATGACTCAG ACCAAGATGG 3082

ATAGGATGGT TAGGGCTTTG CTTCTTGCTG TTTTTCTTTG AACTAGTCAT TGTCCTGCAG 3142

GTCCCTTCAT CTTCCATACC TAGCCCACTC TTTTAGCCCT TACCTTAAAT CTCTCAGATA 3202

AGTTGGTTCA CAAAGAATGT TAAGTACTGA ATCATGTGTG ACTGAGACCA GAGATGGCAA 3262

ATGAATGGCA CACCATTTCT CCTTCTCCTG CCCCAGGGCA GGTACCACTG ATCTGCATCA 3322

GAGTTGCCTG CTATTCTCTG GTGTATCCTT CACATCTAGG TGCCCTCAAG CAGCTGTGTG 3382

AGTGTTGAGA TCTCTGCCAT CTCTGGCTGA GATACTGCTG TCCTGTGAAG TGTTTCCCAT 3442

GACCTTTTTC TTCCCCTTTG AATCCCTCTT GTCTGGAGTA GTCCTTGCCT TCTTCTTGCT 3502

CCAGTAGGCC TTTTCCTTAC CCCAGCCCTT GTGCCAGGCT AAGCTGGTAC AAGAGCTGCC 3562

AACTCACAGA GTTTTGCTAG GCGAGAGAGG TGCAGGGAAG AGGCAGAGGT ATGCACCTTC 3622

CCCCTTGAAG AGAGGGGAAA GGCCTACAGT GGCCCACATA ATTGCCTGAC TCACACTTCA 3682

GCTACCTCTT AATGCCTGTG GAGGGACTGG AGCTGCTGGA TCCCAGTGTG GTGGTGTAGG 3742

AGGCCACAGT GAGCAGGTGG CCCCAGCTGG GTTTCCCAGG TCAGGAATGT GGGCCCCAGG 3802

CAAGGTGCAG CCTTTGCTCA CAGCTCCATC CATGTCTAGA CCTTCAGGCC AGTCTGCAGA 3862

TGAGGTTCCC TACCTTTTTC TTCTCTTCAT TGACCAAATC AACCAATCAC TACAGCTGCT 3922

CTGCTTCTGC TTTCCAAAGT AGCCCAGGTC CTGGGCCAGA TGCAGGGGAG GTGCCTATCC 3982

ATGAGTGAAG GCCAGTGTCT TCCTCACCTG GGTGGTCCCA CACTTGTGAC CCTCAGTTTT 4042

AGGACCCAAG ATCTGTGTTG GTTTCTTAGA TTGCTAGCTT TTCCTCCAGG GGACCACAGC 4102

AGGTGAAGCT CAAGAGCGCA TGGCTCTGCT AATAGTAAAT TGTTTTCAGG GCCTTGTCCA 4162

```
GCTGAGAGCT TCATGTCCAC CAGATTCTGA GAGGTGTCAG CAGCACTTTT TTTTTTTATT      4222

TGTTGTTTGT TTTCCATGAG GTTATCGGAC CATGGGCTGA GCTCAGGCAC TTTCTGTAGG      4282

AGACTGTTAT TTCTGTAAAG ATGGTTATTT AACCCTCCTC CACCCCATCA CGGTGGCCCT      4342

GAGGGCTGAC CCGGAGGCCA GTGGAGCTGC CTGGTGTCCA CGGGGGAGGG CCAAGGCCTG      4402

CTGAGCTGAT TCTCCAGCTG CTGCCCCAGC CTTTCCGCCT TGCACAGCAC AGAGGTGGTC      4462

ACCCCAGGGA CAGCCAGGCA CCTGCTCCTC TTGCCCTTCC TGGGGGAAAG GAGCTGCCTT      4522

CTGTCCCTGT AACTGCTTTC CTTATGGCCC AACCCGGCCA CTCAGACTTG TTTGAAGCTG      4582

CACTGGCAGC TTTTTTGTCT CCTTTGGGTA TTCACAACAG CCAGGGACTT GATTTTGATG      4642

TATTTTAAAC CACATTAAAT AAAGAGTCTG TTGCCTTAAA AAAAAAAAAA AAAAA           4697
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
GTG GAC GTG GAT GAG TAC GAC GAG AAC AAG TTC GTG GAC GAG GAA GAC        48
Val Asp Val Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp
  1               5                  10                  15

GGC GGC GAC GGC                                                      60
Gly Gly Asp Gly
            20
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Glu Glu Glu Glu Asp Asp Asp Glu Asp Glu Asp Glu Glu Asp Asp
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Glu Glu Glu Glu Asp Asp Asp Glu Asp Glu Asp Glu Glu Asp Asp Val
1               5                   10                  15

Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Glu Asp Asp Asp Pro Asp Gly Phe Leu Gly
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Val Asp Val Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp
1               5                  10                  15

Gly Gly Asp Gly Gln Ala Gly Pro Asp Glu Gly Glu Val Asp
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Asp Glu Gly Glu Val Asp
1               5
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Glu Glu Glu Glu Glu Glu Glu Glu Asp Asp Glu Asp Asp Asp Asp Asp
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Glu Glu Glu Glu Glu Glu Glu Glu Asp Asp Glu Asp Asp Asp Asp Asp
1               5                  10                  15

Val Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Val Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Pro Pro Gly Lys Pro Ala Leu Pro Gly Ala
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Glu Asp Gly Val Gln Gly Glu Pro Pro Glu Pro Glu Asp Ala Glu
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
GAA GAG GAA GAA GAT GAT GAT GAA GAT GAA GAT GAA GAA GAT GAT          45
Glu Glu Glu Glu Asp Asp Asp Glu Asp Glu Asp Glu Glu Asp Asp
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 78 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
GAA GAG GAA GAA GAT GAT GAT GAA GAT GAA GAT GAA GAA GAT GAT GTG          45
Glu Glu Glu Glu Asp Asp Asp Glu Asp Glu Asp Glu Glu Asp Asp Val
  1               5                  10                  15

TCA GAG GGC TCT GAA GTG CCC GAG AGT GAC                                 78
Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
GTG TCA GAG GGC TCT GAA GTG CCC GAG AGT GAC                              33
Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
GAG GAT GAT GAC CCC GAT GGC TTC TTA GGC                                  30
Glu Asp Asp Asp Pro Asp Gly Phe Leu Gly
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
GTG GAC GTG GAT GAA TAT GAC GAG AAC AAG TTC GTG GAC GAA GAA GAT          48
Val Asp Val Asp Glu Tyr Asp Glu Asn Lys Phe Val Asp Glu Glu Asp
1               5                  10                  15

GGG GGC GAC GGC CAG GCC GGG CCC GAC GAG GGC GAG GTG GAC                  90
Gly Gly Asp Gly Gln Ala Gly Pro Asp Glu Gly Glu Val Asp
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
GAC GAG GGC GAG GTG GAC                                                  18
Asp Glu Gly Glu Val Asp
1               5
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
GAG GAG GAG GAG GAG GAG GAG GAA GAC GAC GAG GAC GAC GAC GAC GAC          48
Glu Glu Glu Glu Glu Glu Glu Glu Asp Asp Glu Asp Asp Asp Asp Asp
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
GAG GAG GAG GAG GAG GAG GAG GAA GAC GAC GAG GAC GAC GAC GAC GAC          48
Glu Glu Glu Glu Glu Glu Glu Glu Asp Asp Glu Asp Asp Asp Asp Asp
1               5                  10                  15
```

```
GTC GTG TCC GAG GGC TCG GAG GTG CCC GAG AGC GAT                    84
Val Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
GTC GTG TCC GAG GGC TCG GAG GTG CCC GAG AGC GAT                    36
Val Val Ser Glu Gly Ser Glu Val Pro Glu Ser Asp
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
CCC CCC GGG AAG CCA GCC CTC CCA GGA GCC                            30
Pro Pro Gly Lys Pro Ala Leu Pro Gly Ala
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
GAG GAT GGG GTC CAG GGT GAG CCC CCT GAA CCT GAA GAT GCA GAG         45
Glu Asp Gly Val Gln Gly Glu Pro Pro Glu Pro Glu Asp Ala Glu
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Arg Asp Val Ser Glu Glu Leu
1               5
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
CGT GAT GTC TCT GAG GAG CTG                                    21
Arg Asp Val Ser Glu Glu Leu
1               5
```

CLAIMS

1. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:1;

(b) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:2;

(c) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:3;

(d) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:4;

(e) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:5;

(f) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:6;

(g) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:7;

(h) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:8;

(i) a polynucleotide encoding the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:9;

(j) a polynucleotide capable of hybridizing to and which is at least about 95% identical to the polynucleotide of (a)-(h) or (i) wherein the encoded polypeptide is capable of binding to LDL; and

(k) a biologically active fragment of polynucleotide (a)-(i) or (j) wherein the encoded polypeptide is capable of binding to LDL.

2. An isolated polynucleotide of claim 1 wherein said member is selected from the group consisting of:

(a) a polynucleotide encoding the polypeptide comprising the amino acid residues 8-22 (SEQ ID NO:19), 8-33 (SEQ ID NO:20), 23-33 (SEQ ID NO:21) or 208-217 (SEQ ID NO:22) of the amino acid sequence as set forth in SEQ ID NO:7;